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STUDIES OF THE GENUS PHYTOPHTHORA

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INTRODUCTION

From a study of one of the species of Phytophthora it appeared that there was a great need of a comparative study under the same conditions of all the described species of the genus. Hitherto, the various species had never been gathered and grown by any one investigator; nor had comparisons been made when grown under similar conditions; nor had any general survey of the genus been made since it was established, although species had been added from time to time. The cytology of the genus has likewise been almost entirely neglected. As a result of this, together with the imperfections in the descriptions in some cases, it is almost impossible to identify a species with any degree of accuracy. Moreover, because of the scarcity of spore forms on some of the original hosts, as well as their variation when present, herbarium specimens are practically useless for purposes of identification. Another reason which has doubtless tended to check work with this genus is the difficulty encountered in growing some of the species of Phytophthora in culture, even after they have been obtained pure on artificial substrata.

In this group of fungi it is often not sufficient in making transfers to remove a bit of mycelium. The cœnocytic nature of the mycelium necessitates the transfer of an especially long strand so that the cells may remain unbroken. They are so difficult to grow that some of the species, such as *P. thalictri* and *P. agaves*, the latter of which has not yet been described, have not so far been reported in culture.

The object of this paper is to present the results of the writer's studies of the genus from a systematic and a biometrical standpoint. A tenta-

[1] The writer began this work in the Department of Plant Pathology of Cornell University, and completed the major portion of it as a member of the Office of Cotton, Truck, and Forage Crop Disease Investigations. He wishes gratefully to acknowledge the many courtesies shown him by Prof. H. H. Whetzel and Dr. Donald Reddick, in whose laboratories the work at Cornell University was carried on; the valuable suggestions received from Dr. Raymond Pearl, of the Maine Agricultural Experiment Station, and Dr. H. H. Love, of Cornell University, in connection with the biometrical portion of the paper; and the aid given by Mrs. N. E. Fealy, of the Department of Agriculture, in editing the manuscript.

tive table is offered for the separation of species in the hope that it will aid other investigators in identifying the forms with which they work. This paper should be followed by others on parasitism, host relationships, and cytology of all forms that will throw light on the systematic relationship of the species. When these studies are completed, it will perhaps be possible to arrange a more natural classification.

ECONOMIC IMPORTANCE OF THE GENUS PHYTOPHTHORA

Although the actual number of species of Phytophthora is small, geographically they are very widespread, their presence having been recorded from the Tropics as well as the temperate regions, including Japan, India, Java, most of the countries of Europe, Australia, the East Indies, North America, and South America. Botanically, the hosts of *Phytophthora* spp. are distributed among 15 families, ranging from the Pinaceae to the Scrophulariaceae (16)[1], and including plants of all ages and textures. On account of its omnivorous character, the injuries it causes are serious. These injuries include the damping-off of seedlings, spots on leaves, the rotting of fleshy tubers and rhizomes, and cankers on woody stems. The genus has attracted most attention through the attacks of one of the species on the common potato (*Solanum tuberosum*). Although a number of our leading pathologists have devoted their best efforts to the study of this species, considerable work on its life history and cytology still remains to be done.

The genus Phytophthora was founded by De Bary (1) on the potato-blight fungus. Up to the present time Saccardo has enumerated the following species of the genus:

Phytophthora infestans (Mont.) De Bary (1).
Phytophthora cactorum (Cohn and Lebert) Schroeter (31).
[*Peronospora cactorum* Cohn and Lebert (18).
Peronospora fagi Hartig (14, 15), *Peronospora sempervii* Schenk (30), *Peronospora omnivora* De Bary (2)]
Phytophthora phaseoli Thaxter (34).
Phytophthora colocasiae Raciborski (25).

The following species should now be added:

Phytophthora nicotianae Van Breda de Haan (3).
Phytophthora thalictri Wilson and Davis (36).
Phytophthora syringae Klebahn (17).
Phytophthora faberi Maublanc (19).
Phytophthora arecae (Colem.) Pethybridge (23), (24).
Phytophthora parasitica Dastur (10).
Phytophthora erythroseptica Pethybridge (24).

Another species, *P. agaves* Gandara, has been mentioned in literature (13); but as yet no description has been given, and no cultures have been distributed.

[1] Reference is made by number to "Literature cited," pp. 273–276.

As the contributions to the genus in recent years are well reviewed in a paper by Guy West Wilson (37), the writer will not attempt to review them here.

EXPERIMENTAL METHODS EMPLOYED

Pure cultures were obtained from as many of the foregoing species and from as many sources as possible. Of the 11 species[1] described, nine were studied and grown in pure culture. In addition to these, cultures of *P. fagi*, which is given in Saccardo as a synonym of *P. cactorum*, were also obtained. It was not possible to procure fresh material of *P. thalictri* or *P. colocasiae*. The following is a list of the cultures used and sources from which they were obtained:

P. infestans, isolated from potatoes from New York and Maine by the writer, and received from G. P. Clinton, Connecticut, I. E. Melhus, Wisconsin, and S. Ito, Japan.

P. cactorum, isolated by the writer from ginseng from Ohio, Michigan, New York, and Pennsylvania, and by D. L. Peters from a species of Phyllocactus in Germany.

P. phaseoli, isolated by the writer from bean pods from New York, and received from G. P. Clinton, Connecticut.

P. nicotianae, isolated from tobacco in Germany, received from H. H. Whetzel, and from tobacco in Japan, received from S. Ito.

P. syringae, isolated from lilac in Germany, received from H. H. Whetzel, and from lilac in Ireland, received from George H. Pethybridge.

P. arecae, isolated from areca palm in India, received from L. C. Coleman.

P. parasitica, isolated from castor bean, received from J. F. Dastur, India, and G. W. Wilson, New Jersey, the latter culture having probably also come from Dastur.

P. faberi, isolated from cacao in Trinidad, received from J. B. Rorer.

P. jatrophae, isolated by Jensen in Java from one of the nettles and obtained from the Centralstelle für Pilzkulturen, Amsterdam, Holland.

P. fagi, isolated by George H. Pethybridge from young plants of *Fagus* spp. in Ireland, and obtained from Centralstelle für Pilzkulturen, Amsterdam, Holland.

The study of the cultures was supplemented with a study of herbarium material of most of the species.

The ideal method of procedure would have been to study these forms on the original host in each particular case, as in that way no variation in size or form could be attributed to an unnatural substrata relationship; but when the work was begun this was impossible, because the various hosts were not available. The aim was, however, to subject all the forms to identical conditions; and with this in view, a number of artificial media were tried in a preliminary way in order to determine which was most favorable for all the forms.

It was found that the various forms reacted differently on the different media both as regards rate of growth and spore forms produced. The greater number of species made a good growth and produced an abundance of spore forms on oat agar made, with slight modifications, accord-

[1] Prof. S. Ito recently informed the writer that Mr. K. Sawada, of the Formosa Agricultural Experiment Station, lately described in Japanese two new species of Phytophthora occurring on *Allium fistulosum* and *Solanum melogena*, respectively.

ing to the directions given by G. P. Clinton (7). Unless otherwise stated, therefore, this was the media used in all cases where measurements and drawings were made.

In order to obtain a definite spore form, it was necessary in some cases to employ special substrata, such as cooked carrot, potato cylinders, corn-meal agar, and sterilized flies. As artificial media were most generally employed, it seemed important to determine whether these influenced the morphology and size of the various structures to any appreciable extent. Preliminary trials were made with *P. infestans* on potato foliage, but no appreciable differences between the spores produced on this and on those grown on artificial media could be detected. The morphology and size of the various structures of a number of other forms as they occur on the original host are now being studied, and in a later paper these studies will be compared with those recorded here.

The observations and measurements were made at the time of the first appearance of conidia and oospores, at which stage the culture most nearly approaches normal. Soon after this, if the cultures are kept at growing temperatures, the spore forms begin to show various abnormalities, chief among which may be mentioned various proliferations and the formation of secondary conidia.

The temperature at which cultures are grown is a factor in the production of normal and comparable cultures, low temperatures tending to diminish the size of the spore forms and the higher temperatures tending to diminish their production. The cultures from which these studies were made were kept at room temperature varying from 18° to 20° C. No records were kept of the number of times they were transferred. In making the transfers it was found that, if the substrata and temperature conditions were the same, the spore forms appearing were also the same, no matter whether mycelia, conidia, or sexual bodies were used in the transfer.

The purity of the cultures was tested as often as seemed necessary. Several methods were employed in the tests. If the presence of bacteria was suspected or if the sole object was to determine the presence of bacteria, transfers were made from the cultures to nutrient beef agar, a medium on which species of Phytophthora make no growth, but which is especially favorable to the growth of bacteria. In the case of forms which produced an abundance of conidia, dilution plates were made and the cultures recovered from colonies resulting from single conidia. This was a long and tedious process, however, as generally only a small percentage of the conidia germinate on the poured plates. More recently a modification of the above method was used. Where there was a doubt as to purity, the culture was transferred to an Erlenmeyer flask containing media known to produce an abundance of conidia. When the conidia appeared and were in a normal condition, sterilized water was

poured into the flask under aseptic conditions and the culture placed at a temperature favorable for the germination of the conidia by means of swarm spores (20). When such germination took place, each conidium gave rise to from 20 to 40 swarm spores. Poured plates were then made from this solution which contained hundreds of zoospores the majority of which germinated readily on the agar. Instead of obtaining a culture from a single conidium, which is potentially a multiple of zoospores, the new culture was obtained from a single zoospore.

After making a number of measurements of conidia and observing the great variation in size, it occurred to the writer that by continual platings from large selected individuals cultures could be finally obtained which would produce only large conidia, and vice versa. To determine this point, dilution plates were made, a number of single large conidia were marked, and the colonies resulting from one or more of these transferred to agar slants. When these cultures reached the normal condition in the production of conidia, counts were made to determine the percentage of large and small conidia. Dilution plates were again made and the large conidia marked, and the colonies resulting from this second growth or generation were again transferred to agar slants. When the normal spore-producing stage was reached, the percentage of large and small conidia was again determined. This procedure was repeated through five generations, the large conidia being selected in all cases. There was no material difference between the percentage of large and small conidia in the original culture and the culture resulting from the last selection. This indicated that, notwithstanding the great variation in size, the limits and percentages of large and small conidia remain practically constant and are therefore good characters to be employed in taxonomy.

The characters to be relied on in taxonomic work must be constant under all conditions and not correlated with certain adaptations to habitat. While there are advantages in employing characters which show relationship between the species, this is not always possible. In the work here set forth the separation and the relationship of species are made on the aggregate of characters, it being borne in mind that the proportionate value to be attached to each character must necessarily vary.

The most valuable characters that can be employed are doubtless those which are exhibited by the reproductive organs and which, as will be seen, furnish means of separating the genus into different groups. Other characters which may be conveniently used in separating the various species, arranged as nearly as possible in the order of their importance, are (1) the size and morphology of the sexual reproductive bodies, conidia, chlamydospores, and other structures; (2) shape of the terminal papillum in the conidium; (3) the ratio of the length to the width in the conidia; (4) variation in the mycelium; and (5) the microscopic growth, time of appearance of spores, and kind of spore forms produced on various media.

MACROSCOPIC GROWTH ON VARIOUS MEDIA

The media used in making these comparisons were Thaxter's hard potato agar, oat agar, Lima-bean agar, string-bean pods, and corn-meal agar.[1] Various synthetic media were also used; but owing to the small amount of growth produced, they were unsuitable for this work.

Five different series, consisting of three cultures each of the various forms, were made in test tubes and grown at room temperatures. Owing to differences in the hardness of the media, amount of moisture, air, etc., there were slight differences in the amount of aerial mycelium in the different series; but in general the results in all were practically identical and uniform.

As a minor character, the appearance of the macroscopic growth on a given media is of some value when taken with other distinguishing characters. Plantings of the different forms on potato agar poured in large petri dishes, which offer more uniform conditions than can be obtained in test tubes, also showed differences in the luxuriance of growth in some of the species. The distinguishing characters, however, are best shown when grown in test-tube slants. Potato agar was found to be best suited for this purpose. A summary of results of the growth on this medium 12 to 18 days after the transfers were made showed that the cultures may be divided into three groups, as follows: (1) Growth very profuse, aerial, and fluffy. In this group are included *P. parasitica*, *P. fagi*, *P. jatrophae*, *P. cactorum*, and *P. faberi*. (2) Growth less profuse, more or less embedded in substratum, lacking the fluffy appearance, irregular surface, often forming numerous pellicular-like growths and presenting a granular appearance. In this group are included *P. syringae* and *P. nicotianae*. (3) Very little or no growth. In this group are included *P. infestans* and *P. phaseoli*.

On oat agar, unlike the potato agar, *P. syringae* alone could be distinguished at all times from the remaining forms. The character of growth for this form was similar to that on potato agar. All the other forms make a profuse white fluffy growth on the oat agar.

On bean pods no difference could be detected, all forms making a good, fluffy growth except *P. infestans* and *P. phaseoli*, which made little or no growth.

On corn-meal agar no difference could be detected between the different forms, but all made scantier growth than on the other media.

On Lima-bean agar all forms, including *P. infestans* and *P. phaseoli*, made a profuse white growth.

[1] Thaxter's hard potato agar, 200 gm. of potato, 20 gm. glucose, and 15 gm. of agar for every 1,000 c. c. of water; Clinton's oat agar, made according to the directions given by Clinton (8), except that rolled oats and 7½ gm. of agar for every 1,000 c. c. of media were used; bean-pod plugs, consisting of pods of ordinary string beans placed in test tubes with a small quantity of water and sterilized; corn-meal agar, made according to the directions given by Shear and Wood (32); Lima-bean-juice agar, made according to the directions given by Dastur (10).

KIND OF SPORE FORMS PRODUCED AND TIME OF APPEARANCE

Microscopic examinations from time to time of cultures made in test tubes on hard potato agar, oat agar, Lima-bean agar, corn-meal agar, and bean pods, and grown at room temperatures, showed that even when grown under as nearly identical conditions as possible the different cultures did not produce the same spore forms.

As will be seen in Table I, which shows the spore forms found at the end of two and six weeks, certain of the media are more favorable for the production of all the spore forms than others, and oat agar is the most suitable, especially as regards the sexual bodies. It should be borne in mind that the production of the spore forms on the various media shown in the table is not absolute, but what may be expected under normal conditions. This table was compiled as the result of observations during the past three years.

TABLE I.—*Spore forms produced by species of Phytophthora on various media at the end of two and six weeks*

TWO WEEKS

Culture.	Potato agar.	Oat agar.	Lima-bean agar.	Bean pods.	Corn-meal agar.
P. jatrophae	No spores	Conidia; chlamydospores	Conidia; chlamydospores (few).	Chlamydospores (few)	Conidia (few).
P. parasitica	do	Conidia (few)	Conidia	No spores	Conidia (few); chlamydospores.
P. arecae	Conidia (few)	Oospores (few)	Conidia: oospores (few)	No spores	No spores.
P. nicotianae	No spores	No spores	No spores	Chlamydospores (few)	Chlamydospores (few).
P. fagi	Conidia	Conidia; oospores	Conidia; oospores	Conidia: oospores	Conidia (few); oospores.
P. infestans	No spores	Conidia	Conidia	Conidia (few)	Conidia (few).
P. faberi	Chlamydospores	Conidia; chlamydospores	do	Conidia; chlamydospores	Conidia; chlamydospores (?).
P. cactorum from *Panax* sp	Conidia	Conidia; oospores	Conidia; oospores	Conidia; oospores (few)	Conidia (few); oospores.
P. cactorum from *Phyllocactus* sp	do	do	Conidia; oospores (few)	Conidia; oospores	Conidia; oospores.
P. erythroseptica	No spores	Oospores	No spores	Oospores	Oospores.
P. phaseoli	do	Conidia; oospores	Conidia	Conidia	Conidia; oospores (few).
P. syringae	do	No spores	No spores	No spores	No spores.

SIX WEEKS

P. jatrophae	Chlamydospores (few)	Conidia (few); chlamydospores.	Conidia (few); chlamydospores (few).	Conidia; chlamydospores (few).	Chlamydospores.
P. parasitica	No spores	Conidia (few)	Conidia	No spores	Conidia; chlamydospores.
P. arecae	Conidia (few)	Conidia; oospores [a]	Conidia (few); oospores (few).	Conidia (few); oospores (few).	Conidia.
P. nicotianae	Chlamydospores (few)	Chlamydospores (few)	Conidia; chlamydospores (few).	Conidia; chlamydospores	No spores.
P. fagi	Conidia	Conidia (few); oospores	Conidia; oospores	Conidia (few); oospores	Conidia; oospores.
P. infestans	No spores	Conidia; oospore-like bodies [b]	Conidia (few)	Conidia	Conidia.
P. faberi	Conidia; chlamydospores	Conidia (few); chlamydospores.	Conidia; chlamydospores	Conidia; chlamydospores	Conidia; chlamydospores.
P. cactorum from *Panax* sp	Conidia	Conidia; oospores	Conidia; oospores	Conidia; oospores	Conidia (few); oospores.
P. cactorum from *Phyllocactus* sp	Conidia; oospores (few)	do	do	do	Do
P. erythroseptica	No spores	Oospores	No spores	Oospores	Oospores.
P. phaseoli	do	Conidia; oospores	Conidia (few)	Conidia	Conidia (few).
P. syringae	do	Conidia (few)	No spores	Oospores	Conidia.

[a] Numerous oospores were produced on oat agar soon after the culture was received in 1913. Recent examination of transfers from the original culture show very few or no oospores.

[b] See text for discussion of these bodies.

In general the time of appearance of the spore forms from different strains of the same species on a given medium may vary, but eventually the same form appears. The kind of spore forms and the time of their appearance, as in the case of the macroscopic appearance of the growth, may be influenced by the length of time the fungus is kept in culture; and consequently these characters can be of little value in a systematic separation of the species, except when used in conjunction with more stable differences. The production and appearance of certain spore forms in some of the cultures of *P. infestans* will be discussed in a later paragraph.

COMPARATIVE MORPHOLOGY

MYCELIUM

The mycelium of all the species is white and aerial or submerged, as shown by the growth on the artificial substrata previously mentioned, and the hyphæ when young are cœnocytic and filled with a dense, granular protoplasm, oftentimes intermingled with larger particles, probably fat and glycogen. In old cultures, where the supply of nutrition is not so abundant, septation may often be seen; and at this stage the granular protoplasm becomes less dense, and many vacuoles appear. Since the tips of the hyphæ are the young growing parts it would appear that the granules are translocated thither from the older basal portions of the hyphæ.

The appearance of the hyphæ varies, depending on whether the mount was made from aerial or from submerged growth, the aerial mycelium being straight and the submerged much twisted and gnarled.

The mycelia of the different species do not show any distinguishing characters when grown on oat agar. Plate 71, *B*, shows the mycelium of *P. syringae* on this agar, and this is the general appearance of the mycelium of the other forms when grown on this medium.

On potato agar the character of the mycelia is similar to that on oat agar, except in the case of *P. syringae*, in which species the hyphæ present a gnarled and tuberculate appearance (Pl. 71, *A*). This character makes the mycelium of *P. syringae* readily distinguishable from the other forms when grown on potato agar. Ward (35) observed a similar appearance in the mycelium of *Pythium gracile*, and suggested that these structures may serve as reservoirs of protoplasm for the immediate use of the mycelium or for the formation of oogonia. Butler (4) likewise observed structures in the same genus and compared them to the toruloid structures often formed by fungi, the function of which is to carry on a vegetative propagation of the species. Butler's drawings, in his Plate 1, figures 3 and 4, bear a striking resemblance to the figure shown by the writer, to whom these structures appear to be more nearly homologous with sclerotia, though lacking the texture of the latter. The tuberculate protuberances are slightly darker, densely packed with protoplasm, and much more capable

of withstanding adverse conditions than the ordinary hyphæ. These protuberances on the mycelium of *P. syringae* grown on potato agar can be used to advantage in separating it from the other species. It is practically impossible to distinguish any of the other species by their mycelial characters with any degree of accuracy. It should be noted, however, that the mycelium of *P. nicotianae*, whether grown on oat or potato agar, generally contains larger and a greater number of the globoid particles of a fatty or glycogen nature than any of the other species.

The size of the mycelia varies greatly, as shown by measurements made from aerial and from submerged growth. The following are the limits of variation for each of the different forms:

FORM.	SIZE. μ
P. parasitica	1. 91 to 7. 66
P. infestans	2. 87 to 11. 49
P. phaseoli	2. 87 to 11. 49
P. syringae	2. 87 to 7. 66
P. nicotianae	2. 87 to 17. 23
P. jatrophae	1. 27 to 7. 66
P. arecae	1. 91 to 7. 66
P. cactorum	1. 91 to 7. 66
P. faberi	1. 91 to 7. 66
P. fagi	1. 91 to 7. 66
P. erythroseptica	1. 91 to 7. 66

CONIDIOPHORES

As several of the forms produced very few or no conidia-bearing hyphæ on the ordinary agars previously employed, it was necessary to grow some of them on special media in order to study the conidiophores. For example, in the case of *P. erythroseptica*, it was necessary to use flies as the medium in order to obtain conidiophores. Owing to the great length of these structures and the manner in which spores are borne, they can not be studied under high magnification.

The general procedure followed varied according to the form. By means of the following method the majority were easily studied: Van Tieghem cells were carefully cleansed and placed in a large moist chamber and sterilized. Clean cover glasses were flamed, and a drop or two of melted media placed on each and allowed to harden, at the same time all possible precautions being taken to prevent contamination. After this each drop was inoculated with a pure culture of the form to be studied, and the cover glasses then inverted on the Van Tieghem cells, at the bottom of which a few drops of sterile water had previously been placed. The cover glasses were held in place by means of sterilized vaseline. At the end of not less than 36 hours the conidiophores and conidia began to appear and were easily examined with the low magnification of the microscope.

The number of conidia found on a single conidiophore varies from a minimum of 2 to a maximum of more than 20. The sympodial type of branching prevails. Plate 72 shows representative types of conidiophores in the various forms made with the 7.5 ocular and 16 mm. objective. As shown in this plate, such forms as *P. phaseoli* and *P. nicotianae* are more prolific in the production of conidia than others. Examination of the conidiophores of *P. infestans*, *P. phaseoli*, and *P. thalictri* with a higher magnification showed that they differ from the other forms in that there is a slight thickening of the conidiophore immediately below the point of attachment. This character is constant on both the natural substratum and in the artificial cultures, as shown by both *P. infestans* and *P. phaseoli*. The conidiophores vary greatly in size, the variation ranging from 200 to 500 μ in length and 3.5 to 8.5 μ in width.

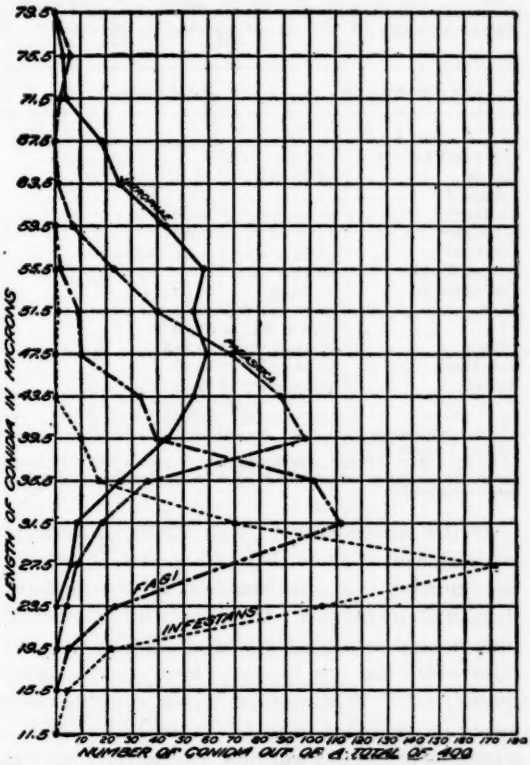

FIG. 1.—Graph showing the variation in length of conidia of *Phytophthora infestans*, *P. fagi*, *P. parasitica*, and *P. jatrophae*.

CONIDIA

Conidia are formed in all the species of Phytophthora studied, and are almost always terminal, although in rare instances structures are seen which may be intercalary conidia. They are elliptical to ovate, approaching the globose, with a more or less prominent apical papillum. In all the forms the color of the conidia is the same—that is, pale gray to colorless. The contents in all cases are finely granular, sometimes with a large central vacuole. The size of the granules and the presence of vacuoles depend on the age of the conidium. As it advances in age the protoplasm and granules appear to divide and become less dense and the number of vacuoles may also increase. Neither the color nor

the contents of the conidia show any distinguishing characters (Pl. 73, 74).

MEASUREMENTS OF CONIDIA.—Measuring spore forms to delineate species in the various fungus groups is a well-established practice. In forms less variable than those of *Phytophthora* spp. a small number of such measurements might be sufficient to determine the average size. The conidia of species of Phytophthora, however, are so variable that a small number of measurements are likely to be misleading. This is brought out by the following figures, which show the variation in the measurements of the same species as given by 12 different workers.

Name of worker.	Size of conidia:	Size of oospores.
	μ	μ
Cohn and Lebert	45 by 35 to 68	20 by 70.
Hartig	25 to 40 by 25 to 40	
Schenk [1]		20 (oogonium).
De Bary	35 to 40 by 50 to 60 to 93	16 to 24.[2]
Schroeter	35 to 40 by 50 to 60	24 to 30.
Osterwalder	14.64 to 24.4 by 119.56	24 (oogonium).
Himmelbauer	Not given	30 to 45.
Zimmerman	17 to 30 by 25 to 60	None found.
Hori	30 to 50 by 50 to 60	26 to 28.
	Abnormal, 29 by 85.5	
Bubak	15 to 25 by 15 to 120	Not given.
Van Hook	30 to 42 by 40 to 58	Do.
Writer	15 to 30 by 18 to 54	21 to 36.

[1] "Die kleinsten derselben sind 5, die grossten 36 Theilstriche meines zeissechen Mikrometers lang und 4 bis 25 Theilstriche breit."

[2] De Bary states that the oospore is in general three-fourths to four-fifths the diameter of the oogonia, and gives 24 to 30 μ as the diameter of the latter, so that the diameter of the oospore given above was obtained accordingly.

As the measurements given above, if correct, would warrant the establishment of more than one species, it seemed advisable to make a large number of measurements of all the forms in culture and present them in their entirety. Accordingly measurements of conidia were made from normal oat-agar cultures of relatively the same age—that is, cultures which had just begun to form conidia, except in the case of four species: (1) *P. erythroseptica*, which was obtained from normal cultures grown on sterilized flies; (2) *P. parasitica*, and (3) *P. nicotianae*, obtained from normal cultures grown on Lima-bean agar, and (4) *P. syringae* from sterilized carrots or bean pods. The scarcity of conidia of these four species on oat agar made the use of special media necessary. It is realized that error may have resulted from the use of different substrata for growing the various forms; but in preliminary trials with a number of different artificial substrata the size of the spore forms remained practically constant on all.

The measurements, of which there were two sets of 200 conidia each, are presented in Table II. These measurements were made at different times and show the variation that may reasonably be expected. The

table gives the class in microns, the number of conidia out of a total of 200, both for length and width, falling into each class, and the results of combining the data of sets A and B. In figures 1 to 6 curves are plotted for the length and width of conidia in microns, and the number of conidia falling into each class out of a total of 400 measurements. These curves were obtained by combining the data given in Table II and making the classes differ by 4 μ instead of 2 μ. They show at a glance the variation that may reasonably be expected in each species,

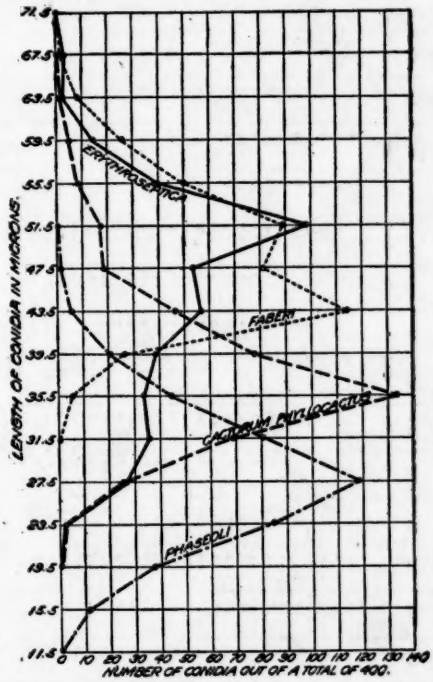

FIG. 2.—Graph showing the variation in length of conidia of *Phytophthora phaseoli*, *P. cactorum* (*Phyllocactus* sp.), *P. faberi*, and *P. erythroseptica*.

as well as the class containing the greatest number of individuals for each species. Further examination of these curves shows that some of them, as, for example, the length of *P. jatrophae*, exhibits a mode of 47 μ and of 55.5 μ. This is probably not to be interpreted as meaning that the normal-sized variation curve is a multimodal one, as too few observations or too numerous classes may cause a similar condition. A similar condition is indicated by the length of *P. nicotianae*. Theoretically all these curves are smooth, and probably would be if an infinite number of measurements were made.

TABLE II.—*Summary of measurements of two sets of conidia of species of Phytophthora*

SET A

Class.	*P. cactorum* (*Panax* sp).		*P. fagi.*		*P. faberi.*		*P. fatrophae.*		*P. nicotianae.*		*P. arecae.*		*P. parasitica.*		*P. infestans.*		*P. phaseoli.*		*P. syringae.*		*P. cactorum* (*Phyllocactus* sp).		*P. erythroseptica.*	
	L.	W.	L.	W.	L.	W.	L.	W.	L.	W.	L.	W.	L.	W.	L.	W.	L.	W.	L.	W.	L.	W.	L.	W.
μ																								
9.5 to 11.49																4		2						
11.5 to 13.49														1		9		2						
13.5 to 15.49														7		5	1	17		2				
15.5 to 17.49		1		4								1		8	4	46	2	29		1				
17.5 to 19.49		3	2	12		1		6				2		23	6	59	10	51		14				
19.5 to 21.49		11	3	19		1		1		2		0		30	10	38	6	37		19				
21.5 to 23.49	2	50	3	28		6		2		10		8	2	44	28	19	16	42	3	24				
23.5 to 25.49	1	30	4	27		4		4	1	6		14	2	22	28	4	17	12	5	24				
25.5 to 27.49	10	52	11	37		16	3	20	1	16	2	31	4	17	51	14	28	7	9	50				
27.5 to 29.49	4	23	14	18		28	0	12	2	16	1	20	3	10	35	1	23	0	4	28				
29.5 to 31.49	20	24	26	25		38	2	22	31	32	7	38	3	15	21	1	35	1	15	22				
31.5 to 33.49	8	3	25	12		12	3	21	7	20	3	23	6	7	10		16		3	5				
33.5 to 35.49	50	2	38	9	4	37	10	38	19	36	12	30	0	9	7		16		15	8				
35.5 to 37.49	3	1	10	5	1	20	9	14	4	10	5	6	8	2			8		8	0				
37.5 to 39.49	44		22	2	5	18	25	28	29	20	37	13	27	4			13		19	1				
39.5 to 41.49	11		2	0	1	13	8	9	16	9	7	5	10	0			3		5	0				
41.5 to 43.49	22		16	2	26	6	25	9	21	10	20	5	20	1			4		23	1				
43.5 to 45.49	13		7		35	0	4	5	8	9	9	3	12				1		9	0				
45.5 to 47.49	2		5		9		21	2	28	3	35	1	33				1		21	0				
47.5 to 49.49	4		3		20		12	2	10	1	13		9						10	0				
49.5 to 51.49	5		6		40		18	4	3		3		19						12	0				
51.5 to 53.49	0		2		20		12	1	9		3		10						5	0				
53.5 to 55.49	0		1		23		20		6		11		17						10	0				
55.5 to 57.49	0				1		5		2		0		3						2	1				
57.5 to 59.49	0				9		11		2		4		4						2					
59.5 to 61.49	0				2		4		0		12		0						2					
61.5 to 63.49	1				3		4		0		5		0						5					
63.5 to 65.49					1		0		1		0		0						1					
65.5 to 67.49							2				1		0						0					
67.5 to 69.49							1				0		0						1					
69.5 to 71.49							1				4		2						2					
71.5 to 73.49											1		0						2					
73.5 to 75.49											1		6						2					
75.5 to 77.49											0								0					
77.5 to 79.49											1								0					
79.5 to 81.49											0								2					
81.5 to 83.49																								

83.5 to 85.49											0								1							
85.5 to 87.49											1								2							
87.5 to 89.49											2								1							

SET B

9.5 to 11.49																1		3								
11.5 to 13.49										1						4		5								
13.5 to 15.49										1						30	2	20		2						
15.5 to 17.49									1	2		7		1		66	6	41		2						
17.5 to 19.49			5						0	16		0		16	3	68	11	68		19						
19.5 to 21.49		8		21					3	17		4		52	2	26	10	34	1	25						
21.5 to 23.49		25	4	50		1		1	9	35		16		56	29	3	30	20	4	26						
23.5 to 25.49	1	34	12	35		4		2	8	22		17		53	19	0	21	6	9	37						
25.5 to 27.49	2	77	19	55		28		9	19	30		48		15	60	2	47	3	17	42						
27.5 to 29.49	6	24	23	15		35	3	5	15	17		18	1	6	27		20		5	23						
29.5 to 31.49	18	23	41	13		42	3	17	20	21	1	41	3	0	29		21		20	18						
31.5 to 33.49	19	4	20	2		20	0	9	14	14	2	12	6	1	10		8		15	3						
33.5 to 35.49	61	2	37	2		28	3	14	29	8	3	11	13		8		12		28	2						
35.5 to 37.49	19	2	16	1		12	3	10	14	5	4	10	15	0	2		8		17	1						
37.5 to 39.49	44	1	11	1	9	20	7	23	20	4	8	8	39		6		3		25							
39.5 to 41.49	6		4		11	4	3	12	10	6	6	2	22		4		1		15							
41.5 to 43.49	11		9		30	4	19	19	12	1	16	5	32						15							
43.5 to 45.49	2		1		23	1	6	12	6		11		24						9							
45.5 to 47.49	6		1		35	0	19	21	10		34		12						12							
47.5 to 49.49	3		1		17	1	7	9	4		18	1	15						4							
49.5 to 51.49	1		1		20		17	19	4		27		7		1				3							
51.5 to 53.49					9		7	3	1		16		4						1							
53.5 to 55.49					15		24	5	1		16		1													
55.5 to 57.49					11		9	5			8		2													
57.5 to 59.49	1				10		19	4			9		1													
59.5 to 61.49					5		9	1			2		2													
61.5 to 63.49					3		15				6		1													
63.5 to 65.49					1		6				4															
65.5 to 67.49					1		11				3															
67.5 to 69.49							4				3															
69.5 to 71.49							2				2															
71.5 to 73.49							1				1															
73.5 to 75.49							2																			
75.5 to 77.49							1																			

SUMMARY OF SETS A AND B

9.5 to 11.49																5		5								
11.5 to 13.49										1				1		13		7								
13.5 to 15.49										1				7		35	3	37		4						
15.5 to 17.49		1		4					1	2		8		9	4	112	8	70		3						7
17.5 to 19.49		3	2	17		1		6	0	16		2		39	9	127	21	119		33		9				24

TABLE II.—*Summary of measurements of two sets of conidia of species of Phytophthora*—Continued

SUMMARY OF SETS A AND B—continued

Class.	*P. cactorum* (*Panax* sp).		*P. fagi.*		*P. faberi.*		*P. jatrophae.*		*P. nicotianae.*		*P. arecae.*		*P. parasitica.*		*P. infestans.*		*P. phaseoli.*		*P. syringae.*		*P. cactorum* (*Phyllocactus* sp).		*P. erythroseptica.*	
	L.	W.	L.	W.	L.	W.	L.	W.	L.	W.	L.	W.	L.	W.	L.	W.	L.	W.	L. t	W.	L.	W.	L.	W.
μ																								
19.5 to 21.49	.....	19	3	40	.....	1	.....	1	3	19	.....	4	.....	82	12	64	16	71	1	44	.....	19	.....	5
21.5 to 23.49	2	75	7	78	.....	7	.....	3	9	45	.....	24	2	100	57	22	46	62	7	50	2	86	2	60
23.5 to 25.49	2	64	16	62	.....	8	.....	6	9	28	.....	31	2	75	47	4	38	18	14	61	0	82	0	5
25.5 to 27.49	12	129	30	92	.....	44	3	29	20	46	2	79	4	32	111	16	75	10	26	92	11	120	12	119
27.5 to 29.49	10	47	37	33	.....	63	3	17	17	33	1	38	4	16	62	1	43	0	9	51	14	39	14	82
29.5 to 31.49	38	47	67	38	.....	80	5	39	51	53	8	79	6	15	50	1	56	1	35	40	42	16	0	0
31.5 to 33.49	27	7	45	14	.....	32	3	30	21	34	5	35	12	8	20	.....	24	.....	18	8	26	11	35	50
33.5 to 35.49	111	4	75	11	4	65	13	52	48	44	15	41	13	9	15	.....	28	.....	43	10	95	9	1	7
35.5 to 37.49	22	3	26	6	1	32	12	24	18	15	9	16	23	2	2	.....	16	.....	25	1	38	5	32	26
37.5 to 39.49	88	1	33	3	14	38	32	51	49	24	45	21	66	4	6	.....	16	.....	44	1	47	3	0	0
39.5 to 41.49	17	.....	6	0	12	17	11	21	26	15	13	7	32	0	4	.....	4	.....	20	0	30	1	38	12
41.5 to 43.49	33	.....	25	2	56	10	44	28	33	11	36	10	52	1	0	.....	4	.....	38	1	29	.....	50	2
43.5 to 45.49	15	.....	8	.....	58	1	10	17	14	9	20	3	36	.....	0	.....	1	.....	18	0	17	.....	6	0
45.5 to 47.49	8	.....	6	.....	44	0	40	23	38	3	69	1	45	.....	0	.....	1	.....	33	0	8	.....	53	1
47.5 to 49.49	7	.....	4	.....	37	1	19	11	14	1	31	1	24	.....	0	.....	.....	.....	14	0	10	.....	0	.....
49.5 to 51.49	6	.....	7	.....	60	.....	35	23	7	.....	30	.....	26	.....	1	.....	.....	.....	15	0	8	.....	58	.....
51.5 to 53.49	0	.....	2	.....	29	.....	19	4	10	.....	19	.....	14	.....	.....	.....	.....	.....	6	0	9	.....	40	.....
53.5 to 55.49	0	.....	1	.....	38	.....	44	5	7	.....	27	.....	18	.....	.....	.....	.....	.....	10	0	5	.....	10	.....
55.5 to 57.49	0	.....	.....	.....	12	.....	14	5	2	.....	8	.....	5	.....	.....	.....	.....	.....	2	1	3	.....	30	.....
57.5 to 59.49	1	.....	.....	.....	19	.....	30	4	2	.....	13	.....	5	.....	.....	.....	.....	.....	2	.....	2	.....	2	.....
59.5 to 61.49	0	.....	.....	.....	7	.....	13	1	0	.....	14	.....	2	.....	.....	.....	.....	.....	2	.....	3	.....	12	.....
61.5 to 63.49	1	.....	.....	.....	6	.....	19	.....	0	.....	11	.....	1	.....	.....	.....	.....	.....	5	.....	1	.....	0	.....
63.5 to 65.49	.....	.....	.....	.....	2	.....	6	.....	1	.....	4	.....	0	.....	.....	.....	.....	.....	1	.....	.....	.....	2	.....
65.5 to 67.49	.....	.....	.....	.....	1	.....	13	.....	.....	.....	4	.....	0	.....	.....	.....	.....	.....	0	.....	.....	.....	2	.....
67.5 to 69.49	.....	.....	.....	.....	.....	.....	5	.....	.....	.....	3	.....	0	.....	.....	.....	.....	.....	1	.....	.....	.....	1	.....
69.5 to 71.49	.....	.....	.....	.....	.....	.....	3	.....	.....	.....	6	.....	2	.....	.....	.....	.....	.....	2	.....	.....	.....	.....	.....
71.5 to 73.49	.....	.....	.....	.....	.....	.....	1	.....	.....	.....	2	.....	0	.....	.....	.....	.....	.....	0	.....	.....	.....	.....	.....
73.5 to 75.49	.....	.....	.....	.....	.....	.....	2	.....	.....	.....	1	.....	6	.....	.....	.....	.....	.....	2	.....	.....	.....	.....	.....
75.5 to 77.49	.....	.....	.....	.....	.....	.....	1	.....	.....	.....	0	.....	.....	.....	.....	.....	.....	.....	0	.....	.....	.....	.....	.....
77.5 to 79.49	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1	.....	.....	.....	.....	.....	.....	.....	1	.....	.....	.....	.....	.....
79.5 to 81.49	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	0	.....	.....	.....	.....	.....	.....	.....	0	.....	.....	.....	.....	.....
81.5 to 83.49	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	0	.....	.....	.....	.....	.....	.....	.....	2	.....	.....	.....	.....	.....
83.5 to 85.49	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	0	.....	.....	.....	.....	.....	.....	.....	1	.....	.....	.....	.....	.....
85.5 to 87.49	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1	.....	.....	.....	.....	.....	.....	.....	2	.....	.....	.....	.....	.....
87.5 to 89.49	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	2	.....	.....	.....	.....	.....	.....	.....	1	.....	.....	.....	.....	.....

In *P. cactorum* isolated from *Phyllocactus* sp. and from *Panax* sp. the greatest number of individuals as regards length and width fall in the same class, although the actual number of species in all the classes varies. The measurements of conidia of *P. fagi* show that it is closely related to *P. cactorum*. There is also a striking relationship between *P. infestans* and *P. phaseoli*.

As shown by the curves in figures 1 to 6, the classes containing the greatest number of individuals in the different species show the least variation for the width of the conidia. The length of the conidia therefore may be adopted arbitrarily to divide the species into two groups: Those in which the predominating class is more than 34.5 μ in length, and which include the forms *P. faberi*, *P. jatrophae*, *P. arecae*, *P. syringae*, and *P. erythroseptica*, and those in which the predominating class is 34.5 μ or less, and which includes the forms *P. cactorum*, *P. fagi*, *P. nicotianae*, *P. infestans*, and *P. phaseoli*.

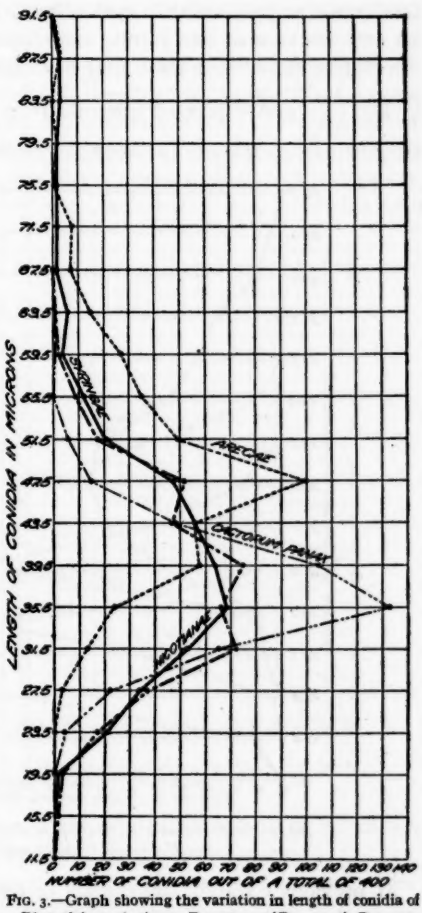

FIG. 3.—Graph showing the variation in length of conidia of *Phytophthora nicotianae*, *P. cactorum* (*Panax* sp.), *P. arecae*, and *P. syringae*.

Differences in length or width alone in different species do not give an accurate idea as to differences in form or shape of the conidia. The form or shape is a constant character and should be employed in differentiating the species. *P. parasitica*, for example, can be readily distinguished from *P. nicotianae* by the fact that the conidia of the former are long and elliptical, while those of the latter are ovate to globose, as shown in Plates 73 and 74. Similar differences exist in the conidia of other species also; but while such differences can be readily detected under the microscope, it has not been possible heretofore to express

them quantitatively. The writer proposes the following method for this purpose:

The differences ranging from ellipsoidal to spherical may be expressed largely as differences between the ratios of the length of the two diameters drawn perpendicular to each other. Thus, in text figure 7 is shown an extreme case of two forms, one ellipsoidal and the other spherical. The difference between these may be indicated by the difference between $\frac{aa}{bb}$ and $\frac{a'a'}{b'b'}$ and expressed quantitatively 2 and 1, since in this hypothetical case aa is twice the length of bb, and bb is equal to $a'a'$ and $b'b'$.

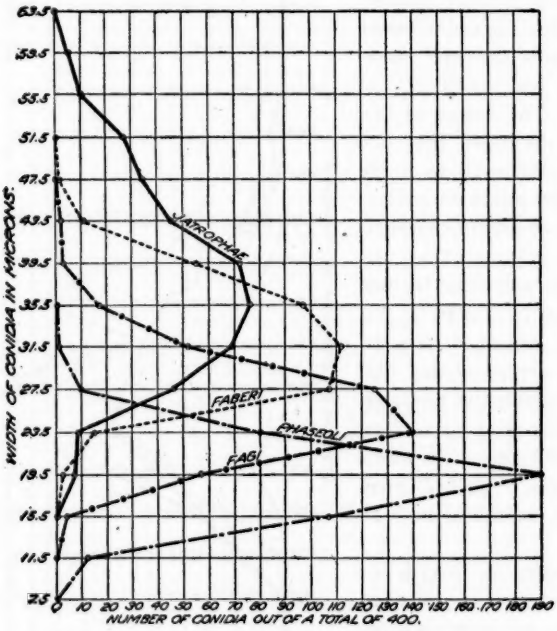

FIG. 4.—Graph showing the variation in width of conidia of *Phytophthora fagi*, *P. phaseoli*, *P. faberi*, and *P. jatrophae*.

As previously stated, the conidia of the *Phytophthora* spp. vary from elliptical to globose; therefore measurements made of each conidium of the long and short diameters, as shown above, will express quantitatively the difference heretofore expressed qualitatively. Such measurements in microns were made from conidia of each species taken at random from normal culture. Four hundred conidia were measured for each species and the ratio of the long and the short diameter of each determined. Figure 8 shows diagrammatically the classification of these ratios. It also shows the predominating ratio in each form, as well as the maximum and minimum limits. From this it will be seen at a glance that the

forms *erythroseptica*, *arecae*, and *parasitica* can be distinguished with ease from the remaining forms by the fact the former have a predominating ratio of more than 1.5 and the latter a predominating ratio of less than 1.5. Of the three forms having a ratio of more than 1.5, the predominating ratio of *P. parasitica* approaches 2, while that of *P. erythroseptica* and *P. arecae* is less than 1.75.

Another distinguishing character is the degree of development of the papilla of the conidia. Camera-lucida drawings of the papilla of typical conidia are shown in text figure 9. The papilla of *P. syringae* and *P. erythroseptica* are broad and flat and not easily distinguished from the curvature of the wall. The papilla of *P. faberi* are raised and very prominent, while the papilla of the other forms show all gradations between

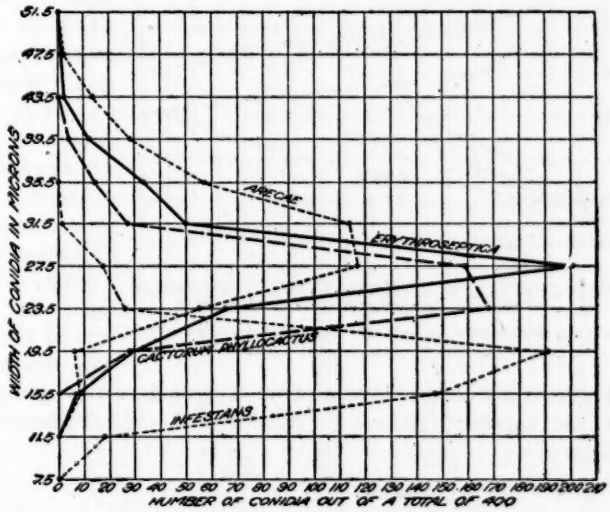

FIG. 5.—Graph showing the variation in width of *Phytophthora infestans*, *P. cactorum* (*Phyllocactus* sp.), *P. erythroseptica*, and *P. arecae*.

these two extremes. The shape of the papilla is constant under all conditions and therefore a good character for use in taxonomy.

GERMINATION OF THE CONIDIA.—In the 10 forms which the writer had in culture, the germination of the conidia was studied; and in all it was found that germination took place either by germ tube or swarm spores, as shown in Plate 75. Potentially each conidium is a sporangium, and, as regards the method by which it germinates, it is influenced, in part at least, by environment.

No differences were noted in the germination by means of germ tubes of the conidia from various species. The method may be described as follows: After the conidium is mature and the papillum formed, one or more tubes are produced. These arise from any point of the conidium,

but most generally near the apex of the spore. The contents of the conidium becomes less dense as a result of passing partly into the growing germ tubes. At the apex the germ tube usually rises from the side of the spore a short distance below the papillum, but seldom from the papillum itself, and in the case of many of these germinations it is very characteristic for the germ tubes to arise in a whorl or cluster near the papillum. As the tubes grow branches are formed.

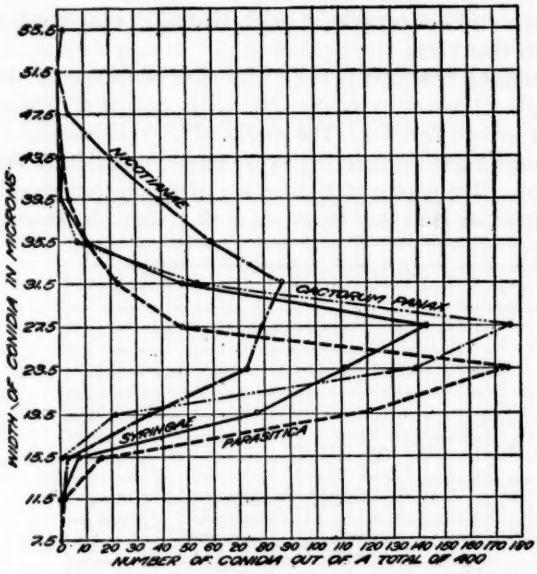

FIG. 6.—Graph showing the variation in width of *Phytophthora parasitica*, *P. syringae*, *P. cactorum* (*Panax* sp.), and *P. nicotianae*.

Swarm-spore germination is common not only to species of Phytophthora, but to some other members of the Peronosporineae. Detailed differences on which separation of genera in this group were based are supposed to exist. Speaking of the Peronosporineae, Butler (4) says:

> The genus Pythium is separated from all the rest by liberating its zoospores in an imperfectly differentiated state into a bladder at the mouth of the sporange, in which differentiation is completed. There are other minor differences, such as the aerial habitat and parasitism of the Peronosporacea, the formation of haustoria correlative with the latter, etc. None of these differences are absolute.

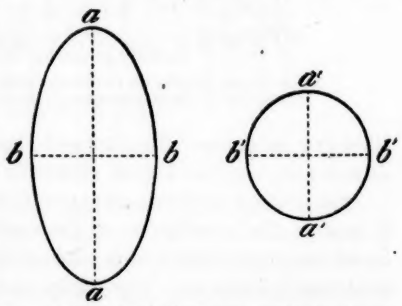

FIG. 7.—Diagrammatic illustration showing that the ratio of the long to the short diameter of ellipsoidal and spherical bodies offers a quantitative means for designating their shape.

In the study of swarm-spore germination in the genus Phytophthora it was found that at least some of the species liberate their zoospore mass into a bladder or vesicle similar to that described for *Pythium palmivorum* (Butler, 5). This shows a still greater relationship to *Pythium*

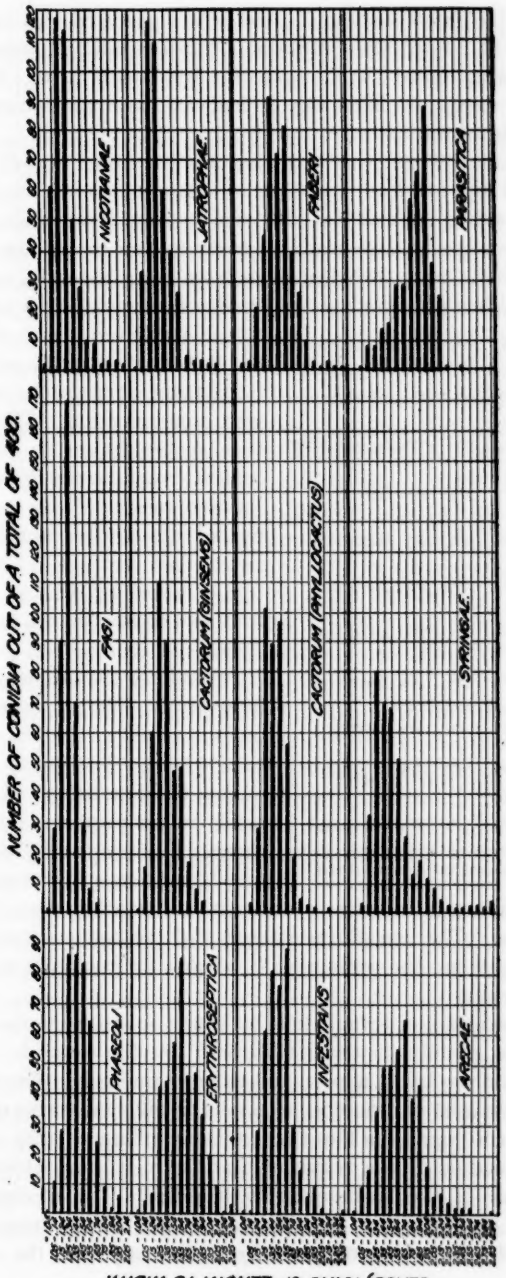

FIG. 8.—Diagram of the arrangement in classes of the ratios of the length to the width of the various conidia, showing the limits of variation and modes.

spp. than has been suspected. The writer observed this bladder-like membrane in *Phytophthora cactorum*, *P. arecae*, and *P. parasitica*. It was previously noted in *P. parasitica* by Dastur (10). Perhaps its evanescent nature explains why it had not been previously observed in some or in all the forms.

Considerable difficulty in obtaining swarm spores at will has been experienced by previous investigators. By means of the following-described method the writer at all times obtained good results: Cultures of the various species were made on media known to produce an abundance of conidia, 250 c. c. Erlenmeyer flasks being generally used for the purpose. When an abundance of conidia were obtained, a small quantity of sterile water was poured into the flask containing the culture, care being taken at the same time to prevent contamination. The flask was then gently shaken to dislodge the conidia from the conidiophores, and the suspension of the spores was poured into another sterilized Erlenmeyer flask and held at a temperature of about 15° C. At the end of from two to five hours a large majority of the conidia had germinated. By examining the solution at various intervals the different stages of germination, as shown in Plate 75, were readily seen. The length of time required for conidial germination depends on temperature conditions, as shown for *P. infestans* by Melhus (20), who found temperatures below 20° C. more favorable for indirect or zoospore germination than the higher temperatures. The age of the culture and the temperature at which spores are kept are the paramount conditions for germination by means of swarm spores.

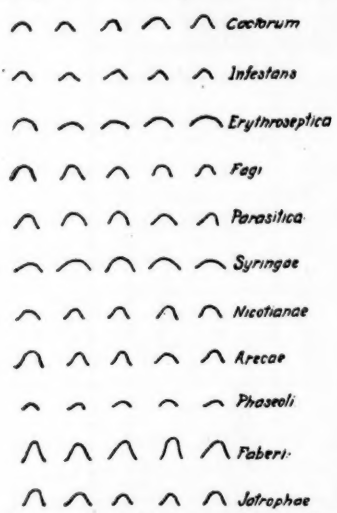

FIG. 9.—Camera-lucida drawings showing differences in the shape of the terminal papilla.

The first evidence of germination by swarm spores is the movement of protoplasmic granules in the sporangium. In the forms in which the vesicles occur the latter appear and enlarge gradually. Careful microscopic examination of a number of condia in different stages of development gives the impression that the terminal papillum enlarges and enters into the formation of the vesicle or that the membrane which lines the inner surface of the sporangium is stretched. The contents of the sporangium gradually pass into the vesicle, and after a time the latter ruptures and sets the swarm spores free. In many cases the vesicle and

its contents are much larger than the original sporangium, and it is difficult to understand how the large swarm-spore mass was contained in the smaller sporangium only a few seconds before. After the liberation of the swarm spores, the vesicles begin to contract, all signs of the opening disappear, and if any zoospores remain they are unable to escape. Plate 75 shows various stages in the germination of *P. arecae* illustrating these points.

When no vesicle is apparent, the process of swarm-spore germination is essentially the same as described, but there is in this case a variation in the manner of emergence of the swarm spores from individual conidia. From the narrow papilla-like opening the swarm spores issue singly or in pairs, the time of emergence varying from 4 to 10 seconds. At times the swarm spores are held together at the opening for 2 to 3 seconds, after which they swim in all directions. Frequently for some reason a few of the swarm spores do not emerge with the majority. For instance, in one case the writer observed some of these swarm spores struggle for 55 minutes to escape, but without success, although at times they were at the very opening. This gave the impression that the opening closed after the majority had escaped. In one particular case the swarm spores finally came to rest and germinated inside the sporangium, the germ tubes extending through the wall. In many cases two of the swarm spores after emergence seemed to stick together two or three seconds, being held by a fine protoplasmic thread or connection, and then each darted off by itself.

The number of swarm spores in a sporangium varies with the size of the latter, and the species are not characterized by a definite number. Neither do the swarm spores show any marked difference in the various species. All are pyriform to kidney-shaped, and each is provided with at least one light-colored spot, probably a vacuole, which is located near the concave side and which appears to be the point of attachment of two flagella. The latter are unequal in size and vary from one-half to twice the length of the body of the swarm spore. After swimming for a time, the flagella having disappeared, the spores finally rest, become round, and germinate by means of tubes. In none of the species of Phytophthora has diplanitism as occurring in related genera been found.

CHLAMYDOSPORES

The term "chlamydospore" is applied to one of the spore forms found in a number of species of Phytophthora. The chlamydospores are spherical, smooth, thick-walled, and brownish to yellow. They differ in size from the conidia and oospores and are formed either terminally or intercalarily. Various investigators have interpreted their function in different ways. When working with *P. faberi*, Von Faber (12), Coleman

(9), and Rorer (27) observed bodies which they took to be oospores. Von Faber states that he found oospores, but failed to find antheridia and oogonia; Coleman that the oospores "almost always fill the oogonial cavity so completely that the oogonial wall can be made out only with difficulty"; and Rorer refers to them as "parthenogenetic oospores."

In a pure culture of *P. faberi* originally obtained from Dr. Coleman an abundance of these bodies were produced. A great deal of time was devoted to their study in fresh as well as in sectioned and stained material. As a result of these studies the writer is convinced that they are not parthenogenetic oospores but are multinucleate vegetative bodies, serving the same function that chlamydospores serve in other groups of fungi. Dastur (10) observed similar bodies, which he termed "resting conidia," in *P. parasitica*, and pointed out that there is little probability of their being parthogenetic oospores. He says:

> They are over twice the size of normal oospores and that from the very beginning of their development they are thick-walled and slightly yellow tinted, while parthenogenetic oospores have their origin in thin-walled and hyaline oogonia, which on failing to come in contact with the antheridia after reaching maturity undergo the same change, at least outwardly, as they would have if they had been fertilized.

The chlamydospores were produced in culture in *P. faberi*, *P. parasitica*, *P. jatrophae*, and *P. nicotianae*. In all of these their general appearance is the same. Their contents are similar to that of the conidia, being granular, with one or more vacuoles, and are illustrated in Plates 76 and 77. It is significant that in all forms which produced an abundance of chlamydospores no oospores were produced in the cultures.

MEASUREMENTS OF CHLAMYDOSPORES.—Although similar in general appearance, the chlamydospores differ in size in the different species, and this difference is constant no matter on what media the culture is grown. As in the case of the conidia, great variation in size occurs within the species; hence, in order to determine the predominating size, as well as distribution in the various classes, 400 measurements of their diameters were made. The results are given in Table III. As will be seen from this table, the chlamydospores may vary in size from 17.5 to 59.5 μ, and *P. faberi* is the only one which has a predominating class of more than 32.5 μ. The difference in size of the predominating class of the chlamydospores of *P. faberi* and of the remaining three is sufficient to be employed in their separation.

TABLE III.—*Summary of measurements of chlamydospores and oospores of species of Phytophthora*

Class.	Chlamydospores.				Oospores.						
	P. faberi	*P. jatrophae*	*P. nicotianae*	*P. parasitica*	*P. cactorum* (*Phyllocactus* sp.)	*P. cactorum* (*Panax* sp.)	*P. fagi*	*P. syringae*	*P. phaseoli*	*P. arecae*	*P. erythroseptica*
μ											
17.5 to 19.49...	0	0	3	0	1	2	0	0	6	0	0
19.5 to 21.49...	0	0	18	0	5	3	2	0	16	0	0
21.5 to 23.49...	0	8	59	5	32	22	13	13	69	8	1
23.5 to 25.49...	0	16	45	17	58	41	14	23	92	9	3
25.5 to 27.49...	0	47	81	46	178	160	67	75	140	37	4
27.5 to 29.49...	4	45	40	58	68	95	72	87	47	45	9
29.5 to 31.49...	23	71	45	121	48	58	106	107	26	84	37
31.5 to 33.49...	25	41	28	59	7	12	51	53	3	67	44
33.5 to 35.49...	51	45	33	43	3	7	46	30	1	59	96
35.5 to 37.49...	52	37	5	20	0	0	13	6	0	37	61
37.5 to 39.49...	80	37	20	19	0	0	12	4	0	31	93
39.5 to 41.49...	45	25	4	6	0	0	2	1	0	8	29
41.5 to 43.49...	63	18	8	3	0	0	1	1	0	6	13
43.5 to 45.49...	19	6	3	2	0	0	0	0	0	6	8
45.5 to 47.49...	20	4	4	1	0	0	0	0	0	2	2
47.5 to 49.49...	11	0	3	0	0	0	0	0	0	1	0
49.5 to 51.49...	7	0	0	0	.....	.....	.....	.....	.....	.....	.....
51.5 to 53.49...	2	0	1	0	.....	.....	.....	.....	.....	.....	.....
53.5 to 55.49...	1	0	0	0	.....	.....	.....	.....	.....	.....	.....
55.5 to 57.49...	0	0	0	0	.....	.....	.....	.....	.....	.....	.....
57.5 to 59.49...	1	0	0	0	.....	.....	.....	.....	.....	.....	.....
Total...	400	400	400	400	400	400	400	400	400	400	400

The curves shown in figures 10 to 12 were constructed from the data given in Table III, except that the classes were made to differ by 4

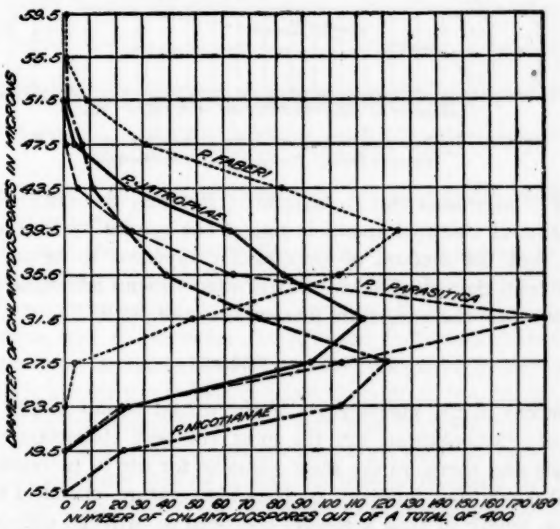

FIG. 10.—Graph showing the variation in the diameters of chlamydospores of *Phytophthora nicotianae*, *P. parasitica*, *P. faberi*, and *P. jatrophae*.

instead of 2 μ. Figure 10 shows the variation in the diameter of the chlamydospores in each of the species, as well as the differences in the

classes containing the greatest number of individuals in each species. Arranged in the order of size, beginning with the largest, we have *faberi*, *jatrophae*, *parasitica*, and *nicotianae*.

GERMINATION OF CHLAMYDOSPORES.—Dastur (10) states that the "resting" conidia do not require a period of rest before germination and that when grown in water germination takes place at the end of 24 hours. After a careful examination of the figures given by Dastur for the "resting" conidia of *P. parasitica* the writer has no doubt that they are identical with those which appeared in his own cultures and which are designated chlamydospores. So far, however, all attempts to germinate these either from young cultures or from cultures of considerable age have failed, as have also all attempts to germinate the chlamydo-

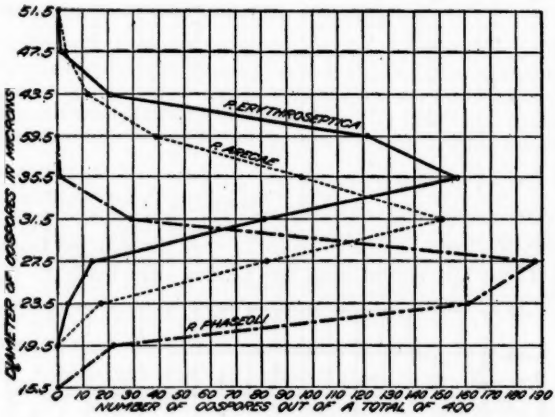

FIG. 11.—Graph showing the variation in the diameters of oospores of *Phytophthora phaseoli*, *P. arecae*, and *P. erythroseptica*.

spores of *P. nicotianae* and *P. jatrophae*. Recently the writer did obtain germination of chlamydospores in one of the species by wintering pure cultures, but the method of germination appeared to be so radically different from that observed by Dastur that it seems advisable to repeat the work before the results are presented to the public.

SEXUAL BODIES

Differences in the morphology of the sexual bodies in the different species of Phytophthora are the most constant characters under all conditions and therefore the most valuable for use in taxonomic work. External conditions, however, such as temperature, chemical nature of substrata, length of time a species has been in artificial culture, abundance of food supply, and may other factors have been known to influence the rapidity of development, as well as the number of sexual bodies produced in culture not only in the genus Phytophthora but in

other members of the Peronosporineae. For example, on its receipt from Dr. Coleman *P. arecae* had produced and for some time afterwards continued to produce an abundance of oospores in culture, but now, after remaining in culture for three years, it produces scarcely a single oospore. Dastur (10) observed this to be the case also in *P. parasitica*. He says: "There is as much variability and uncertainty in the production of these oospores as Clinton found in the production of *P. infestans*." Cultures of this species received by the writer from Dastur in 1914 and grown on media on which sexual bodies were previously reported by him failed to show a single oospore.

What actually determines the production of sexual bodies in *Phytophthora* spp. has not been solved. Some of the species produce these

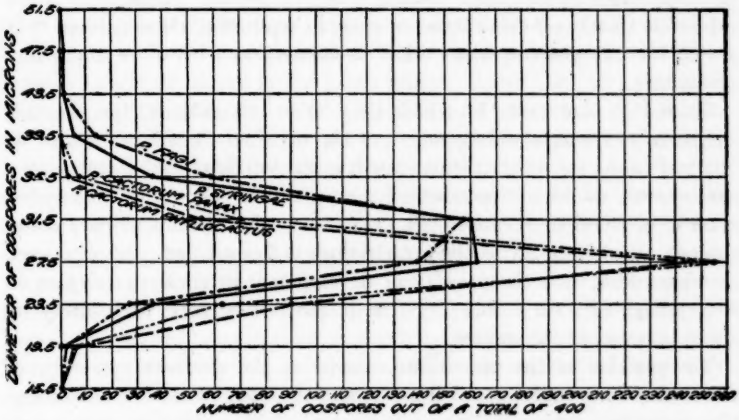

FIG. 12.—Graph showing the variation in the diameters of oospores of *Phytophthora cactorum* (*Phyllocactus* sp.), *P. cactorum* (*Panax* sp.), *P. fagi*, and *P. syringae*.

bodies readily and in great abundance on artificial media, while others produce few or none.

Oogonia and the subsequent development of oospores have been studied by the writer from cultures in *P. cactorum* from both *Phyllocactus* sp. and *Panax quinquefolia*, *P. fagi*, *P. syringae*, *P. phaseoli*, *P. arecae*, *P. erythroseptica*, and *P. infestans*. In addition *P. parasitica* Dastur (10), *P. nicotianae* von Breda de Haan (3), and *P. colocasiae* Butler and Kulkarni (6) are known to produce oospores in culture, but in the case of these three species oospores have not been seen by the writer.

As a result of four years of intermittent work with the various species of Phytophthora some general statements may be made on the behavior of these cultures as regards the development of the sexual bodies. The species *P. cactorum*, *P. fagi*, *P. phaseoli*, and *P. erythroseptica* produced oospores in great abundance on a number of media. In the case of *P. syringae*, *P. infestans*, and *P. arecae* their production requires special

media. Sterilized carrots were employed for *P. syringae*. A slight modification of oat agar as originally used—that is, Quaker-oat agar as used by Pethybridge and containing 7.5 gm. of agar to 1,000 c. c. of media—was employed for the remaining two.

The abundance of oogonia and oospores is so variable that this is a poor character for use in the separation of species. There is one exception, however—that is, *P. phaseoli* as compared with *P. infestans*. Although the former is very closely related to the latter, there is a striking contrast between them in the abundance of oospores produced on oat agar, a large number constantly developing in the case of the former and few or none in the case of the latter. The entire absence of oospores in *P. infestans*, or the scarcity in the development of them, has been observed by all previous investigators working with this species. Although it is not advisable to rely on this character alone in separating these species, it is useful for this purpose when taken in connection with more important characters.

Except in rare cases in which they arise intercalarily, the oogonia originate as terminal enlargements of the mycelial threads, as shown in Plates 76 and 77. In the former case it is often difficult, unless antheridia are present, to determine whether they are true oogonia or chlamydospores, as found in *P. faberi* (Pl. 77, *A*) and other species already mentioned. In certain forms the antheridium is formed first, and in others the oogonium; but the priority of development of these two organs is determined with such difficulty that it can not be used with safety in the separation of the species.

The position of the antheridium varies in the different species, and this variation is constant on all media and can thus be used in separating the genus into groups. These groups may be conveniently designated according to the specific name of one of the species belonging to each group, preferably the oldest described form, as first suggested by Pethybridge (24). The writer has established a third group, which embraces the forms in which the antheridia are entirely absent or in which the relation of the antheridium to the oogonium is unknown. This group is analogous to the Fungi Imperfecti. It will be seen, therefore, that there are three groups, which are designated the "*cactorum* group," the "*phaseoli* group," and the "*faberi* group."

CACTORUM GROUP.—In this group the antheridium, which is elliptical to reniform, is always distinct and is generally on the side of the oogonium. In some stages of development of the antheridium and oogonium a definite fertilization tube can be observed. After fertilization the antheridium disappears entirely or becomes transparent and the contents much reduced. *P. cactorum* (Pl. 77, *C*), *P. fagi* (Pl. 77, *D*), and *P. syringae* (Pl. 76, *A*) are included in this group, and according to Pethybridge probably *P. nicotianae* also. Fertilization in this group consists in a part

of the contents of the antheridium passing through a definite fertilization tube into the oogonium; and following its passage, the consequent changes in the growth of the oogonium into that of the oospore takes place.

PHASEOLI GROUP.—In this group the antheridium, which is not club-shaped, but approaches the form of a flattened sphere, is produced at the base of the oogonium; but no fertilization tube can be observed. After fertilization the antheridium remains attached to the oosphere, and there is very little, if any, change in its contents. In this group are included *P. phaseoli*, *P. arecae*, *P. erythroseptica*, *P. parasitica*, *P. colocasiae*, and *P. infestans* (?). These are shown in Plates 76 and 77. In reality this should be called the "*infestans* group," as *P. infestans* is the oldest described member, but in the opinion of the writer it has not yet been conclusively shown that the method of fertilization in *P. infestans* is the same as in the other members of the *phaseoli* group, and therefore it can logically be placed in the *faberi* group. In all the other members of this group the antheridium develops first and persists throughout the life of the oospore, which is not yet proved to be the case in *P. infestans*.

All previous investigators agree on the general absence of antheridia even in cultures showing all stages of development of the oospore-like bodies, and the author has confirmed their observations. In the few cases where antheridia are apparently present it is hardly safe to say what is the exact method of fertilization. For still other reasons given in subsequent paragraphs, it seemed best to designate this group as above, *P. phaseoli* being second as regards age since its description.

FABERI GROUP.—In this group the antheridia are entirely unknown either in artificial culture or on the natural hosts. It may also include forms in which if the antheridium is present its position, whether basal or side, is not known. It is a tentative group and contains forms which may be subsequently transferred to the other groups. As a result of the present study, it includes *P. faberi*, *P. jatrophae*, and *P. nicotianae*, as shown in Plates 76 and 77, the last form, according to Pethybridge, probably belonging to the *cactorum* group.

Until recently the only method of fertilization in species of Phytophthora was that found in the *cactorum* group, and, as explained, it consists of a fine light thread, or tube, sent out from the antheridium into the oogonium and through which a part of the contents of the former passes into the latter. Following this, the oogonium changes into an oosphere and finally into an oospore. In the forms placed in the *phaseoli* group, however, a new method of fertilization was recently observed. Clinton (7), in speaking of the antheridia and oogonia of *P. phaseoli*, writes:

> For a long time it was difficult to decide whether or not these threads did not actually penetrate the antheridium and grow through it, and we are not yet certain that this does not sometimes occur. Certainly the optical effect is frequently that of an internal thread, with its apical wall very thin as compared with the side walls. * * *. In

time, however, the oogonial thread reaches the top of the antheridium, and curving around its apex begins to swell into the oogonium, which by this time is usually cut off from its basal thread by a septum.

It was Pethybridge (24) who showed that the method of fertilization suggested by Clinton as possibly taking place in *P. phaseoli* actually did occur in *P. erythroseptica*. Dastur (10), working independently, showed that it also occurred in *P. parasitica*. It has since been noted by Butler and Kulkarni (6) in *P. colocasiae*, and Rosenbaum (28) has shown that *P. arecae* agrees with *P. erythroseptica* in this respect. So unique is this method of fertilization and the manner of formation of the antheridium and oogonium in the fungi that the details have not been worked out. Murphy (21), working with Pethybridge and later independently, has shown that a branch destined to become the oogonium grows through the antheridium and forms the oogonium after it emerges. Plate 76, *F*, shows several stages of this process in *P. phaseoli*. The antheridium continues to clasp permanently the stalk of the oogonium. According to Murphy (19), fertilization takes place when

a tube is pushed in from the antheridium through that part of the stalk of the oogonium which lies within the male organ, and through it a single male nucleus and the greater part of the cytoplasm passes in.

Following fertilization, the usual changes, which finally result in the oospore, take place.

In examining species belonging to the *cactorum* group a condition of the sexual organs similar to that found in the *phaseoli* group, which is brought about when the antheridium is superimposed on the stalk bearing the oogonium, is sometimes seen. This condition in *P. cactorum* is strikingly similar to that generally found in *P. arecae* and *P. phaseoli*, but is rare in the *cactorum* group.

Following the discovery of this unusual method of fertilization in some of the species of Phytopthora, Pethybridge (24) proposed the separation of the genus into two genera, the generic name Phytophthora to be retained for the group here designated as the *phaseoli* group, and "Nozemia," a new generic name, for the "*cactorum* group." In reviewing the genus, Wilson (37) suggested that if a new genus is to be established the name "Pleophytophthora," for reasons of priority, should be substituted for "Nozemia."

It does not seem to the writer that the genus Phytophthora should be subdivided into two genera at present, since both the genus Phytophthora and the genus Pythium, which are closely related, need to be investigated further. One of the main differences which has heretofore been supposed to exist between Phytophthora and Pythium is the production in the latter of a sacklike structure into which the swarm spores enter before being liberated. However, it has now been found that this structure is produced in several species of Phytophthora also;

and this, together with other characters, shows a very close relationship between these two genera. What constitutes a character of generic importance is, in the main, relative. It is of paramount importance that further study be made of the species belonging to Phytophthora and Pythium before new genera are established in these closely related groups.

SEXUAL BODIES OF P. INFESTANS

The question as to whether *P. infestans* produced oospores caused much discussion in earlier writings, and among some pathologists and mycologists the feeling that this question is not entirely solved still exists. A brief explanation of the statements regarding the oospore-like bodies of this species previously made in this paper will not be out of place here.

Among the number of strains of *P. infestans* which the writer grew on oat agar in the fall of 1912 one showed a noticeable number of brown, more or less globose bodies, which had every appearance of those described by Clinton (8) as the oospores of *P. infestans*. These differed from the oospores of the other species grown by the writer in that the antheridium was entirely absent in about 95 per cent of the cases examined, the remaining 5 per cent showing attachments which might or might not have been antheridia. These bodies were preserved in permanent mounts, and the many investigators to whom they were shown agreed that they were similar to the bodies described by Clinton and Pethybridge. The writer made diligent search for these bodies in transfers from several of the test tubes in which they appeared in 1912 and in other strains of this fungus, but without success until the fall of 1915, when he resorted to the following procedure:

Inoculations were made on the tops of healthy potato plants with cultures made from the strain of the fungus which originally produced oospores in culture in 1912. After infection took place and a crop of conidia was produced, the conidia were germinated and inoculations again made on potato foliage. In this way there were four separate inoculations and the culture was kept on the potato foliage for approximately a month. Following the last inoculation, the conidia were removed and placed in a small flask of sterilized water, which was kept at a temperature favorable for germination by means of swarm spores. When an abundance of these spores were produced, they were sprayed with a disinfected atomizer on raw potato blocks cut under sterile conditions, and within three days these blocks were covered with a heavy growth of the fungus. A large number of plantings were made from these to oat agar by means of a platinum needle. The first transfers were not free from bacteria, but by carefully watching the cultures and transferring at the proper time to other oat-agar slants a culture free from bacteria was finally obtained. On September 8, transfers from the culture which had recently been growing on

potato foliage were made to oat agar, and when examined 10 days later in a dozen test tubes oospore-like bodies, similar to those previously found by Clinton and Pethybridge, were found in each tube. Another transfer to oat agar from these test tubes produced a supply of oospore-like bodies, but subsequent transfers again gave negative results.

In a communication to the writer under date of June 25, 1915, Pethybridge states that "with *P. infestans*, however, the formation of sexual organs appears to stop after a time. I have not seen any in my cultures for many months now. The cultures, however, are still quite virulently pathogenic to potato tubers." The small number of oospores produced, together with the variability in their production, may explain the failure of so many investigators working with this species to observe these bodies even when grown on a medium favorable for their production.

In the case of *P. infestans*, which has been placed in the *phaseoli* group, Clinton (8) was unable to follow the different steps in the development of the oospores, owing to their scarcity, and claims that the oogonium is much more prominent than the antheridium, "since the latter is so frequently missing." Pethybridge, however, states unhesitatingly that the oospores of *P. infestans* follow the same course of development as in *P. erythroseptica*, and for this reason they are placed in the *phaseoli* group. In all the examinations made by the writer, however, only a few cases were found which might or might not be recognized as antheridia, and with the evidence at hand it does not seem to him that it has been conclusively proved that the oospores of *P. infestans* belong to the *phaseoli* group. On account of the scarcity of antheridia and indefinite knowledge as to their nature it would seem perhaps more logical at present to place them in the *faberi* group or the *phaseoli* group, bearing in mind that their relationship is not yet entirely solved. These are the reasons why the *phaseoli* group is not designated "*infestans* group," and they likewise constitute an additional argument against the subdivision of the genus at present into two genera.

MEASUREMENTS OF OOSPORES.—Four hundred measurements were made of the diameter of the oospores of the various species, and, except in the case of *P. syringae*, cultures of which were made on sterilized carrot plugs, all were made from normal oat-agar cultures. Table III gives the results of these measurements, the first column showing the classes in microns and the other columns the number of oospores out of a total of 400 falling into each class. As it was impossible to obtain a sufficiently large number of oospores in the case of *P. infestans*, this species is not included in the table. The diameter of the oospores, as will be seen, varies from 17.5 to 49.5 μ. The greatest number of oospores of *P. cactorum* from *Phyllocactus* sp. and *Panax* sp. and of *P. phaseoli* fell in the same class—27.5 μ. It should be emphasized, however, that in the case of *P. phaseoli*, unlike that of *P. cactorum*, a larger percentage of the

oospores fall into the classes below 27.5 μ than into the classes above that number. The remaining species have slightly larger oospores.

The two curves in figures 11 and 12 show the diameter of the oospores of the species belonging to the *phaseoli* group, and, as in the case of the curve showing the measurements of the chlamydospores, were constructed from the data given in Table III and the classes made to differ by 4 instead of 2 μ. It will be seen that the oospores of *P. erythroseptica* have the greatest diameter and that those of *P. arecae* and *P. phaseoli* follow. The curves of the species of the *cactorum* group are shown in figure 12, but on account of the smaller variation the differences can not be used to advantage. In the case of *P. infestans* only 150 oospore-like bodies were measured. The results of the measurements of the diameters of these are shown in text figure 13. They varied from 23.5 to 39.5 μ, but most of them fell into the classes ranging from 38.5 to 44.5 μ.

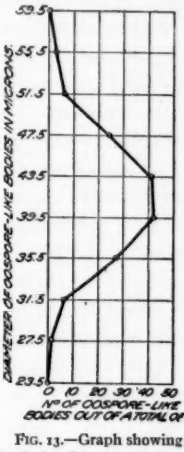

FIG. 13.—Graph showing the variation in the diameter measurements of the oospore-like bodies of *Phytophthora infestans*.

GERMINATION OF OOSPORES.—The methods followed in obtaining germination of oospores are given by the writer in another publication (29). To date *P. cactorum* from *Phyllocactus* sp., *P. cactorum* from *Panax* sp., and *P. fagi* from *Fagus* sp. have been germinated. The process of germination in these three forms is the same. After a period of rest and under proper conditions the oospore wall takes on a striated appearance, and later the striations disappear and the wall itself diminishes in thickness. Through a break in the wall a germ tube is sent out, and into this a part of the contents of the oospore gradually passes. When the tube has reached a certain size, it bears one or more conidia, and these also may germinate by means of germ tubes or by swarm spores.

BIOMETRICAL CONSTANTS

The use of biometry in obtaining biological data has been employed since the middle of the nineteenth century, but few biometrical studies have been attempted with fungi. On account of their variability, the spore forms of the genus Phytophthora offer a good object for such a study.

Pearl (22) says:

> A description which says nothing about the magnitude of the thing described is not complete, but on the contrary, lacks an element of primary importance * * * it is certain that not only are quantitative methods needed in biology, but also that a far more serious need is for something of the methodological viewpoint.

The biometrical methods therefore furnish a means of extending the descriptive method and of expressing quantitatively what investigators have heretofore attempted to express qualitatively.

The constants here obtained, offering a more ready comparison than the mass of individual measurements previously given, are (a) the mean or the point about which the individuals of the group cluster; (b) the median, the point on either side of which exactly half of the individuals fall; (c) the mode—that is, the class containing the greatest number of individuals; (d) standard deviation, expressing the degree of variation in the group; (e) skewness, which is a measure of "the degree of symmetry of the distribution of the individuals composing the group."

In the calculation of the constants the ordinary biometrical methods as given by E. Davenport (11) were followed, and for the moments Sheppard's corrections (33) were employed. In calculating the mode, median, skewness, and probable errors the formulæ given by Pearl and Surface (22) were used.

The writer is aware that the peculiar substrata on which some of the cultures were grown may affect the constants. It may be possible to give in a subsequent paper the results of measurements obtained under normal conditions—that is, directly from the host plants. The constants, together with their probable errors for the conidia of the various species, are shown in Table IV. In the examination of the table the following points should be noted: The species *P. cactorum* isolated from *Panax* sp. and *Phyllocactus* sp. show a remarkable uniformity. *P. fagi* is closely related to *P. cactorum*, the mean for length being slightly greater in the latter. The skewness for both length and width, as shown by *P. fagi*, is more closely related to the strain of *P. cactorum* obtained from *Panax* sp. than from *Phyllocactus* sp. *P. infestans* and *P. phaseoli* can not be distinguished by differences in size of the conidia. *P. jatrophae* and *P. nicotianae* are here shown to differ as regards both length and width. As previously pointed out, there are slight differences in the mycelium of the two species, the latter being somewhat broader and more gnarled and containing a larger amount of a substance of a fatty or glycogen nature. *P. infestans* and *P. erythroseptica*, both of which are parasitic on the potato (*Solanum tuberosum*), show great differences in size. The constants obtained for the standard deviation, as seen, show that the deviation from the mean for length varies from $4.31 \pm 0.102\ \mu$ in the case of *P. infestans* to as high as $11.10 \pm 0.264\ \mu$ in the case of *P. syringae*, and for width the deviation varies from $3.03 \pm 0.072\ \mu$ in the case of *P. infestans* to $8.27 \pm 0.197\ \mu$ in the case of *P. jatrophae*. Skewness in the case of length is positive in all cases, with the exception of *P. jatrophae*, *P. erythroseptica*, and *P. nicotianae*. In the case of width it is positive in all cases with the exception of *P. cactorum* (*Panax* sp.) and *P. syringae*.

Where the skewness is positive, the curve is more spreading on the side of large-sized individuals than in the opposite direction. In general this would point to the fact that the same conditions which favor the production of large individuals in the class as a whole is also more favorable to the production of exceptionally large individuals than exceptionally small ones.

TABLE IV.—*Constants for length and width of conidia of Phytophthora spp.*

LENGTH

Species.	Mean.	Median.	Mode.	Standard deviation.	Skewness (approximate).
	μ	μ	μ	μ	μ
P. fagi	33.65±0.210	33.19±0.264	32.28	6.23±0.148	0.0219±0.041
P. phaseoli	27.87± .195	27.31± .245	26.19	5.77± .137	.2912± .036
P. jatrophae	49.65± .328	49.79± .413	50.08	9.73± .232	−.0447± .041
P. cactorum (*Panax* sp.)	36.62± .181	35.46± .228	33.14	5.37± .128	.6478± .027
P. cactorum (*Phyllocactus* sp.)	37.69± .233	36.03± .293	32.70	6.91± .164	.7724± .025
P. erythroseptica	44.85± .303	45.88± .380	47.93	8.98± .214	−.3432± .035
P. arecae	47.92± .327	46.83± .411	44.65	9.70± .231	.3370± .035
P. syringae	39.86± .374	38.50± .470	35.79	11.10± .264	.3663± .035
P. infestans	27.08± .145	26.78± .183	26.19	4.31± .102	.2054± .029
P. parasitica	43.64± .271	42.88± .341	41.36	8.05± .191	.2833± .037
P. faberi	48.52± .198	48.09± .250	47.23	5.88± .140	.2193± .038
P. nicotianae	37.58± .274	37.62± .344	37.70	8.12± .193	−.0148± .041

WIDTH

P. fagi	25.67±0.146	25.47±0.185	25.08	4.33±0.103	0.1351±0.040
P. phaseoli	19.05± .105	18.86± .134	18.48	3.12± .075	.1826± .039
P. jatrophae	37.55± .279	36.92± .350	35.67	8.27± .197	.0227± .041
P. cactorum (*Panax* sp.)	26.06± .109	26.09± .139	26.16	3.24± .077	−.0324± .041
P. cactorum (*Phyllocactus* sp.)	25.72± .120	25.57± .153	25.27	3.57± .085	.1244± .040
P. erythroseptica	27.65± .177	27.16± .223	26.20	5.25± .125	.2750± .037
P. arecae	30.05± .186	29.85± .234	29.45	5.50± .131	.1090± .040
P. syringae	25.33± .152	25.61± .192	26.18	4.50± .107	−.1900± .039
P. infestans	18.27± .102	18.05± .130	17.61	3.03± .072	.2181± .038
P. parasitica	23.39± .147	22.74± .186	21.45	4.37± .104	.4428± .033
P. faberi	32.29± .159	31.40± .201	29.63	4.73± .113	.5615± .029
P. nicotianae	29.95± .230	29.84± .289	29.63	6.81± .162	.0463± .041

Means and standard deviation for the diameters of the chlamydospores and oospores are given in Table V. The smallest mean of the diameter of chlamydospores is that of *P. nicotianae*, with a standard deviation of 6.03±0.144 μ, while *P. faberi* has the largest mean diameter, with a standard deviation of 4.97±0.118 μ. *P. faberi* can well be separated from the three other forms producing chlamydospores by its larger chlamydospores. The differences in size of the oospores are less striking than those of the conidia, and the variations, as seen from the standard deviation, are likewise less. It has already been pointed out that *P. phaseoli*, *P. arecae*, and *P. erythroseptica* can be separated from the remaining forms in this table by morphological differences in the oospores, skewness being positive in all the forms with the exception of the oospores of *P. syringae*.

TABLE V.—*Constants for chlamydospores and oospores of species of Phytophthora*

CHLAMYDOSPORES

Species.	Mean.	Median.	Mode.	Standard deviation.	Skewness.
	μ	μ	μ	μ	μ
P. faberi	38.98±0.167	38.62±0.211	37.90	4.97±0.118	0.2173±0.0386
P. jatrophae	32.89± .180	32.13± .228	30.61	5.36± .128	.4257± .0332
P. nicotianae	28.83± .203	27.35± .256	24.39	6.03± .144	.7383± .0254
P. parasitica	31.15± .132	30.72± .167	29.86	3.92± .093	.3293± .0359
OOSPORES					
P. cactorum (*Phyllocactus* sp.)	26.78±0.081	26.67±0.104	26.45	2.40±0.057	0.1374±0.0401
P. cactorum (*Panax* sp.)	27.36± .084	27.15± .108	26.73	2.50± .060	.2576± .0377
P. fagi	30.22± .125	30.10± .159	29.86	3.71± .088	.0970± .0407
P. syringae	29.50± .108	29.54± .137	29.62	3.19± .076	−.0376± .0412
P. phaseoli	25.55± .088	25.74± .113	26.12	2.60± .062	.2191± .0386
P. arecae	32.42± .152	32.01± .193	31.19	4.53± .108	.2751± .0374
P. erythroseptica	35.78± .127	35.70± .161	35.54	3.77± .090	.0636± .0410

The ratios of the length to the width of the 400 conidia measured are arranged into classes in figure 8. By the use of these data, representing 400 ratios of length to width of conidia, the means and the standard deviation of the ratios for each species were determined. The results are shown in Table VI. From this it will be clearly seen that the ratio of the length to the width of the conidia can be made use of in the separation of the species; and, in fact, it is suggested that a similar system of measurements in other fungi may perhaps be made use of in delineating species.

TABLE VI.—*Constants as calculated from class ratios of length to width of 400 conidia of species of Phytophthora as shown in figure 9*

Species.	Means.	Standard deviation.	Species.	Means.	Standard deviation.
	μ	μ		μ	μ
P. fagi	1.25±0.004	0.110±0.003	*P. infestans*	1.45±0.006	0.189±0.005
P. phaseoli	1.40± .006	.170± .004	*P. parasitica*	1.82± .009	.259± .006
P. jatrophae	1.28± .006	.168± .004	*P. cactorum* (*Phyllocactus* sp.)	1.39± .005	.149± .004
P. cactorum (*Panax* sp.)	1.34± .006	.165± .004	*P. faberi*	1.47± .007	.197± .005
P. erythroseptica	1.57± .008	.229± .005	*P. nicotianae*	1.25± .006	.171± .004
P. arecae	1.59± .009	.278± .007			
P. syringae	1.49± .011	.314± .007			

Reitz and Smith (26) make the following statement regarding the significance of probable errors:

> In the comparison of two statistical results, the difference between the two results compared to its probable error is of great value. In general, we may take the probable error in a difference to be the square root of the sum of the squares of the probable errors of the two results.
>
> If the difference does not exceed two or three times the probable error thus obtained, the difference may reasonably be attributed to random sampling. If the difference between the two results is as much as five to ten times the probable error, the probability of such differences in random sampling is so small that we are justified in saying that the difference is significant. In fact, a difference of ten times its probable error is certainly significant in so far as there is certainty in human affairs.

TABLE VII.—*Summary of differences in the means of the conidia of species of Phytophthora*

Species.	Difference in means.		Difference in means divided by probable error of difference.	
	Length.	Width.	Length.	Width.
	μ	μ	μ	μ
P. nicotianae and *P. jatrophae*	12.07±0.427	7.60±0.361	28.27	21.05
P. parasitica and *P. arecae*	4.28± .425	6.66± .237	10.07	28.10
P. fagi and *P. cactorum* (*Phyllocactus* sp.)	4.04± .314	.05± .188	12.87	.26
P. infestans and *P. erythroseptica*	17.77± .336	9.38± .204	52.89	45.98
P. infestans and *P. phaseoli*	.79± .243	.78± .146	3.25	5.34
P. cactorum (*Panax* sp.) and *P. cactorum* (*Phyllocactus* sp.)	1.07± .295	.34± .162	3.63	2.10
P. parasitica and *P. erythroseptica*	1.21± .407	4.26± .230	2.97	18.52
P. arecae and *P. erythroseptica*	3.07± .446	2.40± .257	6.88	9.34
P. syringae and *P. cactorum* (*Phyllocactus* sp.)	2.17± .441	.39± .194	4.92	2.01

The probable errors in the differences of the statistical results are shown in Tables VII and VIII. Examination of these tables shows that the differences in length of conidia of *P. nicotianae* and *P. jatrophae*, *P. parasitica* and *P. arecae*, *P. fagi* and *P. cactorum*, and *P. infestans* and *P. erythroseptica* are, without doubt, significant. On the other hand, the figures confirm the previous observation and statement that the pairs *P. infestans* and *P. phaseoli*, *P. cactorum* from *Panax* sp. and *P. cactorum* from *Phyllocactus* sp., *P. parasitica* and *P. erythroseptica* can not be distinguished by their differences in length.

TABLE VIII.—*Summary of differences in the mean diameter of oospores and chlamydospores of species of Phytophthora*

OOSPORES

Form.	Difference in mean diameter.	Difference in mean diameter divided by probable error of difference.
	μ	μ
Arecae and *erythroseptica*	3.35±0.198	16.92
Cactorum (*Panax* sp.) and *coctorum* (*Phyllocactus* sp.)	.59± .117	5.04
Phaseoli and *arecae*	6.87± .176	38.92
Fagi and *cactorum* (*Phyllocactus* sp.)	3.44± .149	23.09
Syringae and *cactorum* (*Phyllocactus* sp.)	2.72± .135	20.15

CHLAMYDOSPORES

Nicotianae and *jatrophae*	4.05± .272	14.89
Jatrophae and *faberi*	6.09± .246	24.76
Faberi and *parasitica*	7.83± .209	37.46

The difference between the pairs *P. arecae* and *P. erythroseptica* and *P. syringae* and *P. cactorum* may or may not be due to random sampling. As regards width, *P. nicotinae* and *P. jatrophae*, *P. parasitica* and *P. arecae*, *P. parasitica* and *P. erythroseptica*, and *P. infestans* and *P. erythroseptica* are certainly different; differences which may or may not be significant are found between *P. infestans* and *P. phaseoli*, and *P. arecae* and *P. erythroseptica;* and finally the differences between *P. cactorum* from *Panax* sp. and *P. cactorum* from *Phyllocactus* sp., *P. fagi* and *P. cactorum*, and *P. syringae* and *P. cactorum* are of no value.

A study of Table VIII shows the differences in the means of the oospores of the various species to be significant and also confirms the identity of *P. cactorum* from *Panax* sp. and from *Phyllocactus* sp. The differences in the mean diameter of the chlamydospores, as given in this table, are likewise significant.

IDENTIFICATION AND SYSTEMATIC SEPARATION OF SPECIES

In determining a species belonging to the genus Phytophthora, which was one of the objects of this study, the following points should be noted, and it is suggested that as many of these as possible be used in the descriptions:

(1) Relation of antheridium to oogonium, whether basal or side.

(2) Shape of terminal papilla and their relation to the curvature of the body.

(3) Measurements of length and width of conidia, and the diameter of chlamydospores and oospores (at least 200 individuals should be measured).

(4) Arrangement of these measurements into classes and determination of the various biometrical constants.

(5) Calculations of the mean length and width of conidia and mean diameter of chlamydospores and oospores.

(6) Ratios of length to width of conidia measured and arrangement of these into classes, showing the mode.

(7) Mean, as determined from ratios of length and width.

(8) Character of conidiophores.

(9) Germination of conidia, character of swarm spore, etc.

(10) Growth on various media at room temperature, and noting macroscopic appearance and spore forms produced at the end of two and six weeks.

(11) Miscellaneous, such as peculiarities in mycelium.

It should be borne in mind that no definite criteria are known for the identification of species of some of the genera closely related to Phytophthora, among which may be mentioned Pythium, Pythiacystis, Peronospora, Plasmopara, and Sclerospora. Until further studies have been made, however, the above points can be employed to good advantage in identifying the species of these genera also.

The following tentative table is offered for the separation of species:

A. CACTORUM GROUP.—Majority of the oogonia have the antheridium on side. *P. cactorum, P. fagi, P. syringae, P. nicotianae.*

B. Apical papilla not prominent, but broad and flat.

C. Mean length of conidia 39.86 μ, mean width 25.33 μ, mode for width 26.18 μ, mean diameter of oospore 29.5 μ.....1. *P. syringae.*

BB. Apical papilla prominent.

C. Chlamydospores absent, mode for length of conidia 32 to 33 μ, mode for width of conidia 25 to 26 μ.

D. Mode for diameter of oospores 29.86 μ, mean diameter 30.22 μ.....2. *P. fagi.*

DD. Mode for diameter of oospores 26 μ, mean diameter 27 μ.....3. *P. cactorum.*

CC. Chlamydospores produced abundantly, mode for length of conidia 37 to 38 μ, mode for width 29 to 30 μ.

D. Mode for diameter of chlamydospores 28.86 μ, mean diameter 31.15 μ, mean ratio of length to width of conidia 1.25.....4. *P. nicotianae.*

AA. PHASEOLI GROUP.—Majority of oogonia have the antheridium at the base. *P. phaseoli, P. erythroseptica, P. arecae, P. parasitica,* and *P. infestans.*

B. Mean ratio of length to width of conidia greater than 1.75.

C. Mean length of conidia 43.64 μ, mean width 23.39 μ, mean diameter of chlamydospores 31.15 μ....5. *P. parasitica.*

BB. Mean ratio of length to width of conidia less than 1.75.

C. Conidia small, mean length less than 30 μ, mean width less than 20 μ.

D. Producing an abundance of oospores on oat agar, antheridia present.....6. *P. phaseoli.*

DD. Oospores or oospore-like bodies lacking or very scarce on oat agar and when present having antheridia entirely absent or indefinite. 7. *P. infestans.*

CC. Conidia large, mean length more than 30 μ, mean width more than 20 μ.

D. Apical papilla not prominent, but broad and flat.

E. Mean diameter of oospore 35.78 μ with a standard deviation of 3.77, mode for length of conidia 47.93 μ, mode for width of conidia 26.20 μ.....8. *P. erythroseptica.*

DD. Apical papilla not as above but prominent, mean diameter of oospore 32.42 μ with a standard deviation of 4.53, mode for length of conidia 44.65 μ, mode for width of conidia 29.45 μ. 9. *P. arecae.*

AAA. FABERI GROUP.—Antheridium entirely unknown or its relation to the oogonium not yet determined, chlamydospores absent or present. *P. faberi, P. jatrophae.*

B. Chlamydospores large, mean diameter more than 35 μ.

C. Mean diameter of chlamydospores 38.98 μ, mean ratio of length to width of conidia 1.47.....10. *P. faberi.*

BB. Chlamydospores small, mean diameter less than 35 μ.

C. Mean diameter of chlamydospores 32.89 μ, mean ratio of length to width of conidia 1.28.....11. *P. jatrophae.*

SUMMARY

Prior to these studies no definite criteria for the identification and separation of the species belonging to the genus Phytophthora were known. With a view to supplying information for this purpose, 11 out of 13 described species were collected and studied from pure cultures grown on artificial media and from herbarium material. The results of these studies may be summarized as follows:

(1) As regards rate of growth and spore forms produced, the various species reacted differently on the different media.

(2) The temperature at which cultures are grown is a factor in the production of normal and comparable cultures.

(3) For the purpose of testing the purity of the cultures from time to time two methods the details of which are described on pages 236–237 were devised.

(4) Continual culturing from large selected conidia does not eventually produce a culture with a predominance of large individuals, or vice versa.

(5) The separation and relationship of species should be made on the aggregate of characters, it being borne in mind that the proportionate value to be attached to each character must necessarily vary.

(6) As a minor character, the macroscopic growth on a given medium is of some value.

(7) The time of appearance of the spore forms from different strains of the same species on a given medium may vary, but eventually the same forms appear.

(8) On oat agar the mycelia of the various species can not be distinguished with any degree of certainty. On potato agar *P. syringae* can be distinguished from the remaining species by the fact that it produces characteristic tuberculate mycelia (Pl. 71, *A*); and likewise *P. nicotianae* can, to a certain extent, be distinguished by the more gnarled mycelia and greater abundance of globoid particles of a fatty or glycogen nature within the threads.

(9) Measurements of the conidia can be employed as an aid in delineating species provided a sufficiently large number are measured. It is suggested that at least 200 should be measured and the different measurements grouped into classes.

(10) For the purpose of obtaining a quantitative measure of the shape of the conidia the ratio of the length to the width should be ascertained and the ratios likewise grouped into classes. A comparison of the conidia of *P. parasitica* and *P. nicotianae* illustrated this point. Heretofore the differences in shape of the conidia of these two species would be expressed only qualitatively, the former being called long and ellipsoidal and the latter short and globose. As a result of measuring the length and width of 400 individuals of each species and obtaining the ratios of the length to the width, the conidia of *P. parasitica*, expressed quanti-

tatively, group themselves about a predominating ratio of 2, while those of *P. nicotianae* are found at 1.2 (fig. 8). Similarly the ratios of the conidia of all the remaining species vary from 1 plus to 2 plus (fig. 8).

(11) The degree of development of the papillum is a good character to be employed in taxonomic work (fig. 9).

(12) In their germination of conidia by means of swarm spores at least some of the species of Phytophthora liberate their zoospore mass into a bladder or vesicle, thus showing a greater relationship to *Pythium* spp. than had been suspected (Pl. 75).

(13) Certain species of Phytophthora produce chlamydospores either terminally or intercalarily.

(14) The measurements of the chlamydospores can be used to good advantage in delineating species. A sufficiently large number should be measured, as in the case of the conidia.

(15) The relation of the antheridium to the oogonium—that is, whether produced at the base or on a side—can be used in separating the genus into groups.

(16) An additional group (*faberi* group), analogous to the Fungi Imperfecti group and embracing forms in which the antheridia are absent or their relation to the oogonium yet unknown, is tentatively established.

(17) In one of the several strains of *P. infestans*, oospore-like bodies, resembling those observed by other investigators, were produced, but antheridia were absent or, if present, were of a doubtful nature.

(18) As in the case of conidia and chlamydospores, a sufficiently large number of measurements of the oospores should be made if the measurements are to be used in identifying a species.

(19) On account of the variability in the size of the spore forms belonging to the genus, a more ready comparison can be made by the use of biometrical constants than by a mass of individual measurements, and such biometrical constants were calculated and arranged into tables. By the use of these constants the identity and relationship of the species are further confirmed.

(20) There being no known criteria for the separation and identification of species in closely related genera, such as Pythium, Pythiacystis, Peronospora, Plasmopara, and Sclerospora, most of the points enumerated in this paper can be employed to good advantage for this purpose in connection with these genera.

(21) A tentative table, employing some of the above characters for the separation of the species, is given on page 271.

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PLATE 71

A.—Mycelium of *P. syringae* grown on potato agar. Note the tuberculate appearance of the mycelium. ×320.

B.—Mycelium of *P. syringae* grown on oat agar. This is the general appearance of the mycelia of the other forms when grown on this medium. ×320.

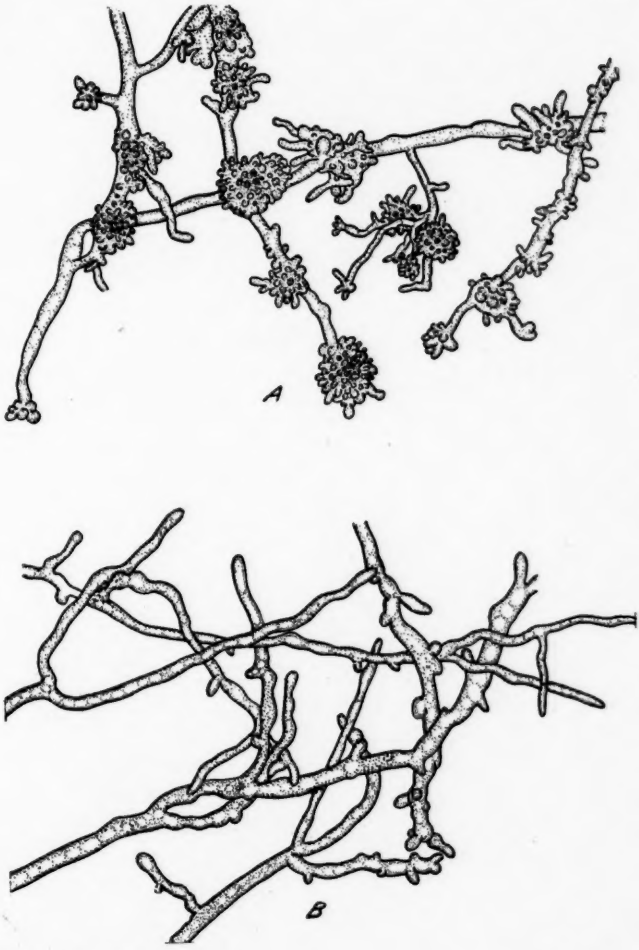
A
B

PLATE 72

PLATE 72

Conidiophores of the various species of Phytophthora studied from Van Tieghem cells. ×54.

A.—*P. jatrophae.*
B.—*P. arecae.*
C.—*P. nicotianae.*
D.—*P. phaseoli.*
E.—*P. cactorum* (*Panax* sp.).
F.—*P. fagi.*
G.—*P. cactorum* (*Phyllocactus* sp.).
H.—*P. faberi*

PLATE 73

Conidia of the various species of Phytophthora illustrating differences in shape of conidia and appearance of the terminal papilla. ×320.

A.—*P. erythroseptica.*
B.—*P. fagi.*
C.—*P. arecae.*
D.—*P. phaseoli.*
E.—*P. parasitica.*
F.—*P. infestans.*

A
B
C
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E
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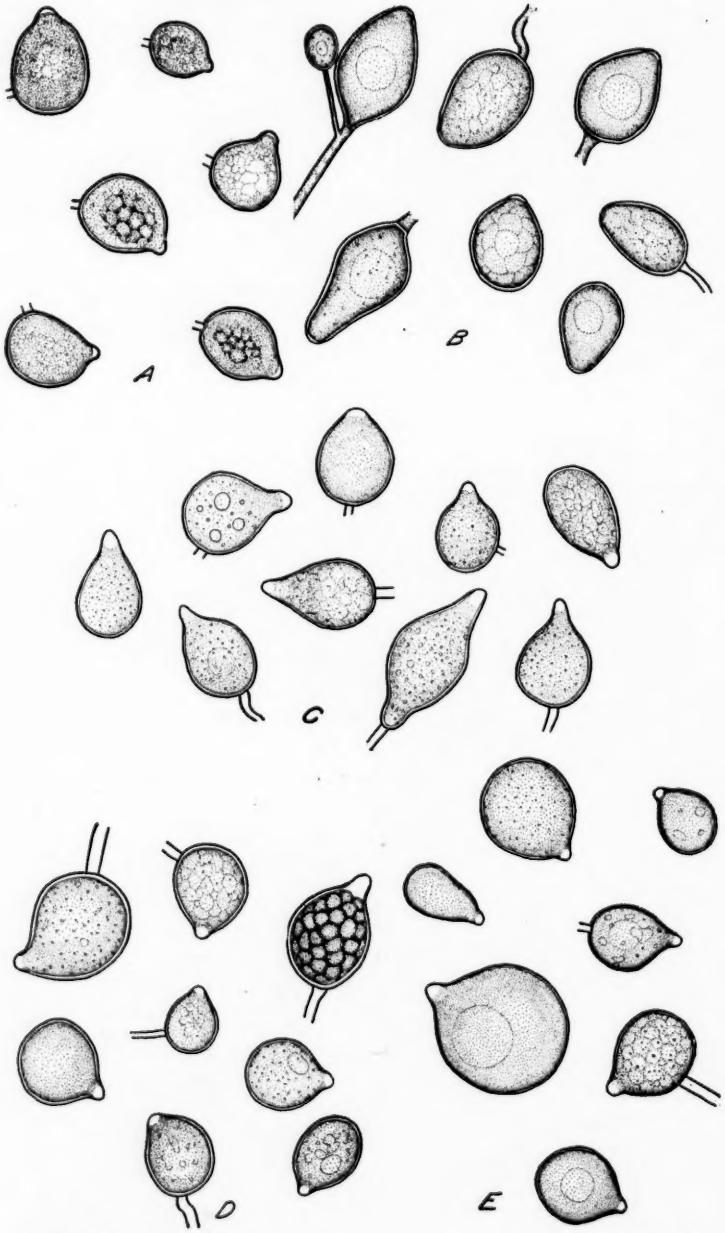
A
B
C
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E

PLATE 74

Conidia of the various species of Phytophthora illustrating differences in shape of conidia and appearance of the terminal papilla. ×320.

A.—*P. cactorum* (*Phyllocactus* sp.).
B.—*P. syringae.*
C.—*P. faberi.*
D.—*P. nicotianae.*
E.—*P. jatrophae.*

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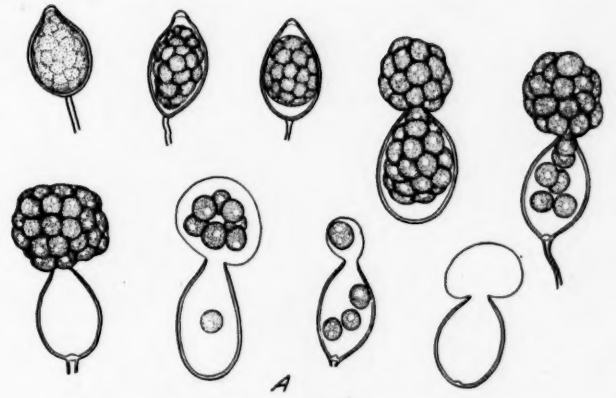
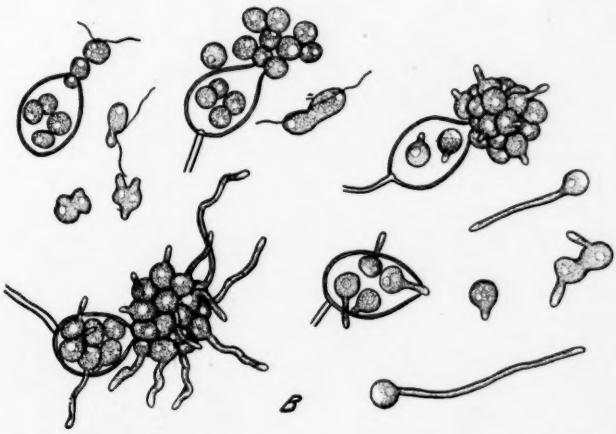
PLATE 75

Stages in the germination of the conidia and swarm spores of *P. arecae*. ×320.
A.—Earlier stages.
B.—Later stages, showing the escape of the swarm spores and their germination.


A


B

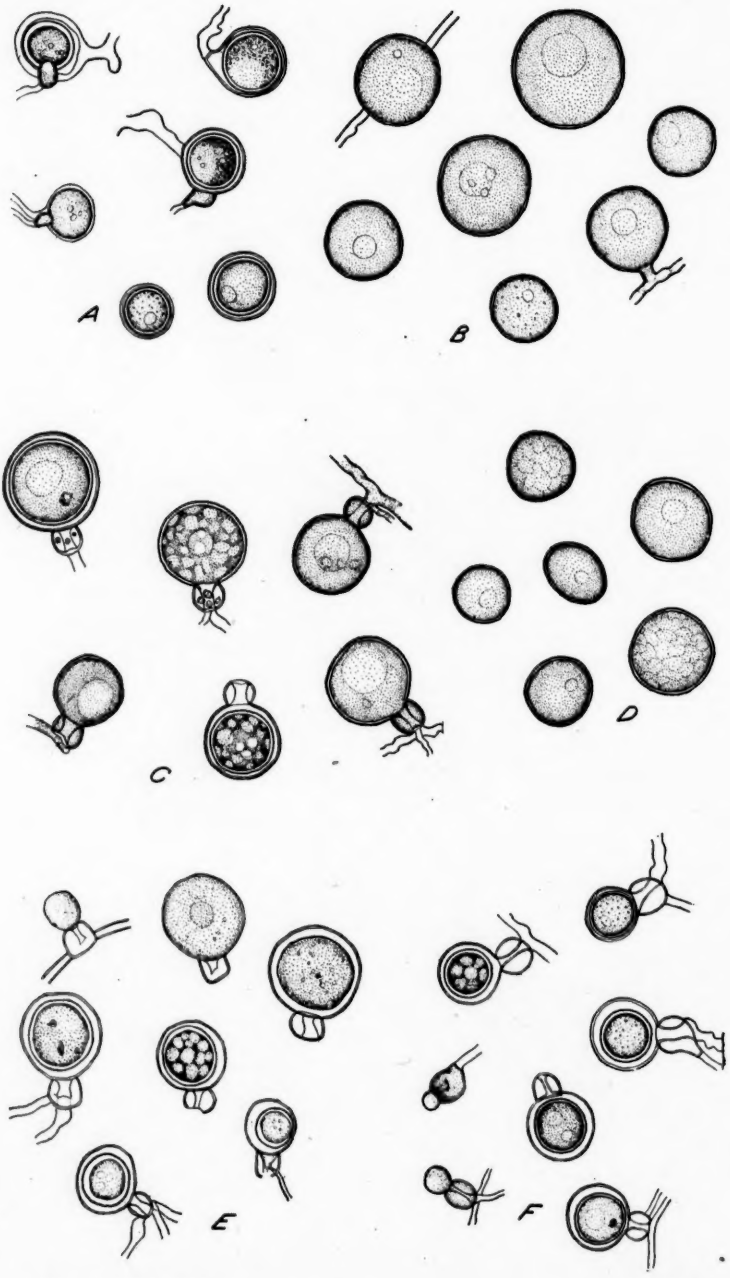

A
B
C
D
E
F

PLATE 76

Chlamydospores and oospores of the various species of Phytophthora, illustrating shape and relation of the antheridium to the oogonium. ×320.

A.—Oospores of *P. syringae.*

B.—Chlamydospores of *P. jatrophae.*

C.—Oospores of *P. arecae.*

D.—Chlamydospores of *P. parasitica.*

E.—Oospores of *P. erythroseptica.*

F.—Oospores of *P. phaseoli.*

PLATE 77

Chlamydospores and oospores of the various species of Phytophthora, illustrating shape and relation of the antheridium to the oogonium. ×320.

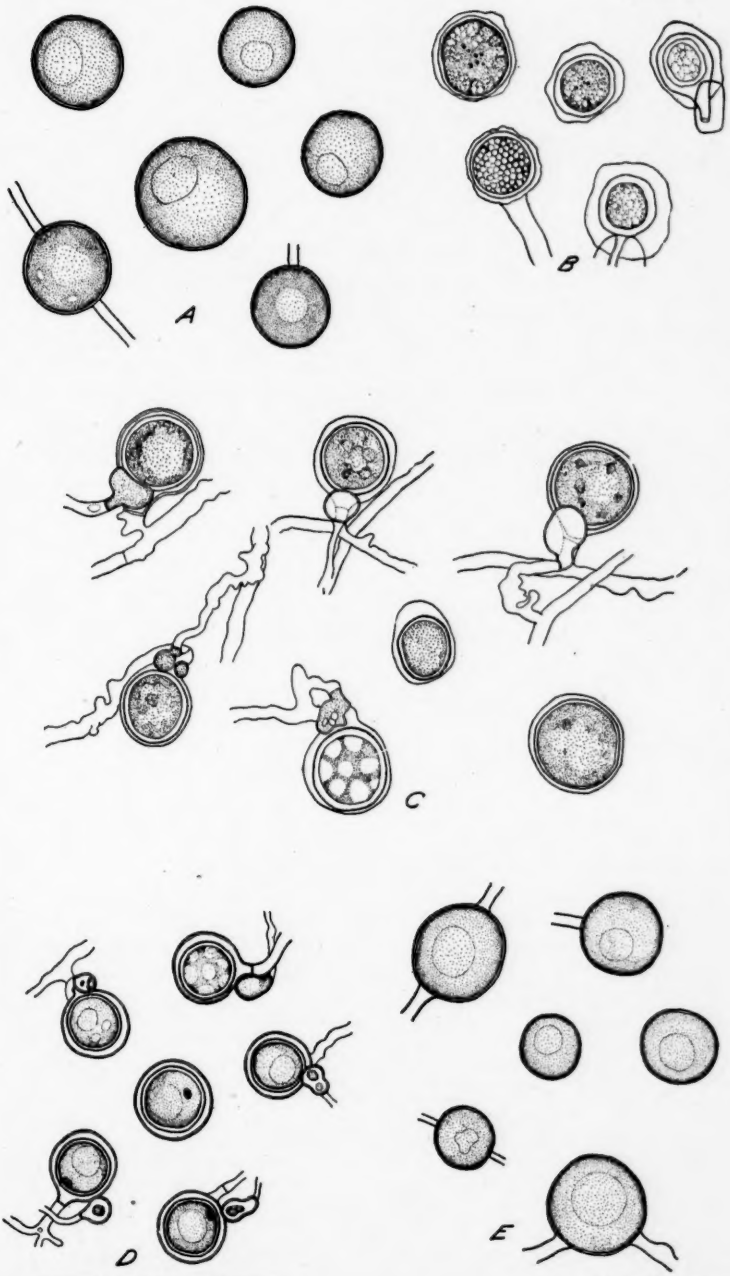
A.—Chlamydospores of *P. faberi*.

B.—Oospore-like bodies of *P. infestans*.

C.—Oospores of *P. cactorum* (*Phyllocactus* sp.).

D.—Oospores of *P. fagi*.

E.—Chlamydospores of *P. nicotianae*.


A
B
C
D
E

TETRASTICHUS BRUCHOPHAGI, A RECENTLY DESCRIBED PARASITE OF BRUCHOPHAGUS FUNEBRIS

By THEODORE D. URBAHNS,

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INTRODUCTION

Tetrastichus bruchophagi Gahan was one of several new species of parasites found attacking *Bruchophagus funebris* Howard, which was breeding in alfalfa seeds throughout the San Joaquin and other valleys of California during the seasons of 1912 and 1913. Infested alfalfa seeds (*Medicago sativa*) were obtained at different times from various localities, and these showed that this parasite attacked *B. funebris* over a widely distributed area.

Most of the observations concerning the habits of this parasite were made from material taken to the temporary laboratory at Glendale, Cal.

DISCOVERY OF THE INSECT AND ITS DISTRIBUTION

This species was first reared by the author in November, 1912, from *B. funebris* infesting alfalfa seeds which were collected at Tulare, Cal. On April 22, 1913, it was secured from material collected at Corcoran, Cal., and on July 9, from Glendale, Cal. It was reared on August 12 of the same year from Yuma, Ariz.; on August 26 from Sacramento, Cal.; and on October 11 from Dos Palos, Cal. During 1914 it was also reared, on June 16, from Stockton, Cal.; September 8, from Red Bluff, Cal.; and September 16, from Woodland, Cal., from alfalfa seeds collected at these points.

On September 11 it was reared from *B. funebris* infesting red-clover seeds (*Trifolium incarnatum*) taken at Albany, Oreg. During April, 1915, it was found on *B. funebris* in alfalfa seeds from Bishop, Cal., and was reared from red-clover heads infested by *B. funebris*, secured through Mr. W. J. Phillips at Charlottesville, Va. On June 10 it was reared from infested red-clover heads secured from Mr. J. J. Davis at La Fayette, Ind., and on July 8 it was reared from red-clover heads secured from Akron, Ind. Mr. C. N. Ainslie reared miscellaneous insects from red clover collected at Hudson, Mich., August 21, 1906, and from Rochester, Minn., August, 1907, among which this species was found.

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Adults of a species of Tetrastichus, of which the specific determination was not given, have been reported as having been reared, together with *B. funebris*, from red clover collected as follows:

E. G. Titus, March 20, 1905, Daggett, Mich.
F. M. Webster, May 24, 1906, Chambersburg, Pa.
F. M. Webster, May 24, 1906, Milton, Pa.
F. M. Webster, January 24, 1906, State College, Pa.
F. M. Webster, January 31, 1906, Lebanon, N. H.
F. M. Webster, January 22, 1906, Tyngsboro, Mass.
W. J. Morse, May 24, 1906, Burlington, Vt.
W. B. Hall, March 19, 1906, Wakeman, Ohio.
G. I. Reeves, January 22, 1907, Pine City, Minn.
J. B. Weems, June 14, 1906, Crewe, Va.
J. L. Phillips, April 5, 1905, Blacksburg, Va.
W. J. Phillips, November 8, 1904, Bonsack, Va.
J. L. Phillips, August 30, 1905, Fort Wayne, Ind.
G. I. Reeves, December 9, 1904, Lincoln, Nebr.
L. Bruner, March 1, 1905, Lincoln, Nebr.
W. J. Phillips, October 21, 1906, Richmond, Ind.
G. I. Reeves, November 1, 1906, Marquette, Mich.
G. I. Reeves, November 1, 1906, Chatham, Mich.
G. I. Reeves, November 3, 1906, Sault Ste. Marie, Mich.
G. I. Reeves, November 24, 1906, Corning, N. Y.
C. V. Piper, September, 1907, Turkestan, Asia.
C. N. Ainslie, October, 1907, Jefferson, Ohio.

CLASSIFICATION AND DESCRIPTION

Tetrastichus bruchophagi Gahan belongs to the hymenopterous superfamily Chalcidoidea, family Eulophidae, and subfamily Tetrastichinae. It was described as a new species by Mr. A. B. Gahan,[1] of the Bureau of Entomology, from type specimens reared by the writer from *B. funebris* infesting alfalfa seeds at Corcoran, Cal. Mr. Gahan's description follows.

Tetrastichus bruchophagi. Female.—Length 1.8 mm. Antennal pedicel and the three funicle joints subequal in length, the club about as long as the two last funicle joints combined; head not wider than the thorax; malar space long, equaling or nearly equaling the height of the eyes; whole head finely lineolated with a few round punctures on the cheeks; prothorax finely punctured; mesoscutum and scutellum finely lineolated, the parapsidal grooves deep and broad, the median line of the mesoscutum distinct but fine; two longitudinal grooves on the mesoscutellum very distinct, the distance between them not equal to half the length of the scutellum; metanotum about half as long as the propodeum and faintly sculptured; propodeum with faint subreticulate sculpture similar to that of the metanotum, the median carina distinct; abdomen conic ovate, as long as head and thorax.

Color dark blue-green; antennæ very dark brownish, the apex of scape below, and underside of pedicel paler; all coxæ, trochanters, and femoræ greenish black; apices of all femoræ, all tibiæ, and the tarsi, except apical joint, pale yellow.

Male.—Similar to the female except for secondary sexual characters.

Type locality.—Corcoran, Cal.

[1] Gahan, A. B. New Hymenoptera from North America. *In* Proc. U. S. Nat. Mus., v. 46, p. 431–443, pl. 39. 1914.

STAGES OF HOST SHOWING PARASITISM

Tetrastichus bruchophagi is parasitic upon the larval stage of its host, *B. funebris*. This parasite attacks the half-grown or fully developed host larva, and after attaching itself punctures the host larval skin, feeding upon the body contents. It is normally an external parasite but in several instances has been found to be completely inclosed within the host larva.

One hundred and eleven larvæ of *T. bruchophagi* under observation showed 106 of these parasite larvæ as externally parasitic and 5 as internally parasitic upon their respective host larvæ. All of the specimens counted in this test were reared to the adult stage for determination. This species has not been observed to be parasitic upon the pupal stage of its host.

HIBERNATION

This species hibernates within infested seeds of alfalfa in which it has attacked its host, and spends the winter in the larval stage. Larvæ becoming fully developed as early as August and September frequently go into hibernation. In a few instances this species was observed to enter the pupal stage late in the fall and to pass through the winter in this stage. Most of the pupæ and undeveloped larvæ are killed by the first severe frost in the fall.

APPEARANCE IN THE FIELDS

Tetrastichus bruchophagi is probably the first of the parasites of *B. funebris* to appear in the fields in early spring. A comparative study of larvæ of this species, together with other parasites of *B. funebris*, shows that the larvæ of *T. bruchophagi* are among the first to transform to the pupal stage and emerge as adults with the approach of warm spring weather. The adults may be seen among the first blossoms of alfalfa and are active over the first developing seed pods.

Table I shows the emergence of adults of *Tetrastichus bruchophagi*, reared from larvæ which had hibernated through the winter; and Table II the emergence of adults of the summer generations as they appeared in the laboratory, from alfalfa seed infested with *B. funebris* and taken from the fields.

TABLE I.—*Emergence of adults of Tetrastichus bruchophagi from hibernated larvæ*

Period of emergence.	Male.	Female.
Mar. 1–15	.....	.....
Mar. 16–31	3	1
Apr. 1–15	6	1
Apr. 16–30	10	6
May 1–15	7	17
May 16–31	21	31
June 1–15	5	52
June 16–30	18	109
July 1–15	4	48
July 16–31	1	2
Aug. 1–15	1	.....
Total	76	267
Percentage of sexes	22.15	77.8

TABLE II.—*Emergence of adults of the summer generations of Tetrastichus bruchophag as they appeared in the laboratory, from alfalfa seed infested with B. funebris and taken from the fields*

Period of emergence.	Male.	Female.
July 1–15	1	20
July 16–31	47	417
Aug. 1–15	71	243
Aug. 16–31	3	72
Sept. 1–15[1]	.....	.....
Sept. 16–30[1]	.....	.....
Oct. 1–15[1]	.....	.....
Oct. 16–31	8	12
Nov. 1–15	4	6
Nov. 16–30	.....	.....
Total	134	770
Percentage of sexes	14.82	85.17

[1] Adults emerged freely, but counts of sexual forms were not made.

OVIPOSITION

This minute parasite searches over the green alfalfa seed pods for those containing seeds infested by *B. funebris*. When a suitable place is found for oviposition, the female suddenly lowers the tip of her abdomen to the surface of the seed pod and forces the ovipositor into the seed pod and infested seed, placing an egg upon the larva of its host. The time required for oviposition varies from about 20 seconds to 1 minute.

LARVA

DEVELOPMENT.—The larva of *T. bruchophagi* upon hatching from the egg finds itself upon the host. In the course of one or two days it takes a more or less permanent position and begins to feed. A very rapid growth follows the first feeding of this parasite. In the course of its develop-

ment indistinct molting is apparent, and fragments of the cast-off larval skin may be noticed peeling off of the developing larva. The fully developed larva has frequently been found to be almost inclosed within the old larval skin. In other cases this cast-off skin has been observed to be worked back over the body of the larva.

DORMANT PERIOD.—After the larva has become full grown, further development toward the pupal stage depends largely upon external climatic conditions to which the seed is subjected. Complete drying of the seed containing this parasite delays pupation, and, in fact, may produce a dormant period to the larval stage of indefinite duration, or until both humidity and temperature are favorable for further development. This dormant period frequently begins in early summer and carries the species to the following year before further development takes place. Material kept in the laboratory showed adults of this species emerging almost two years after the seeds containing the parasite larvæ had been collected in the field.

DESCRIPTION.—The larva (Pl. 78, *A*) varies in color from clear white to smoky white, and some of them have been found to be almost cream colored. They average 1.3 mm. in length and 0.66 mm. in diameter. The general shape is elliptical when at rest. At times the larva takes on a pear-shaped form. It has a small head and 13 body segments. The first three body segments back of the head are slightly wider than the others. The body is free from pubescence and smooth, very lightly segmented. The mandibles are very small and almost invisible.

LENGTH OF LARVAL STAGE.—The length of the larval stage depends greatly upon the duration of the dormant period into which it enters, but under the most favorable conditions this period does not require more than about 10 days and pupation follows soon after the larva is fully developed.

PREPUPAL STAGE.—Before entering the pupal stage, a prepupal period of about 48 hours' duration occurs. During this time the formation of the pupa within the larval skin takes place and the larva loses its normal shape.

PUPA

PUPATION.—After the pupa (Pl. 78, *B*) has formed within the larval skin, the latter breaks open along the dorso-anterior margin and is worked back until it is free from the body of the pupa.

DESCRIPTION.—The pupæ measured averaged 1.2 mm. in length and 0.7 mm. in width. At first the pupa is white, but later the eyes take on a brown color and the body becomes black. The body of the pupa is rather straight, with head bent slightly forward. The appendages are folded close to the body and the pupa is covered by a thin pupal skin. Before changing to the adult stage the pupa becomes almost black, with a blue-green metallic luster.

LENGTH OF PUPAL STAGE.—The pupal stage seems to vary greatly, depending upon temperature conditions of the season in which pupation occurs. Larvæ which had hibernated through the winter went into pupation as early as March, in California, and some of the hibernating larvæ under less favorable conditions did not enter pupation until two or three months later.

Fifty-two males reared from hibernating larvæ showed an average of 17.7 days in the pupal stage. Fifty-six females reared from hibernating larvæ showed an average of 19.7 days in the pupal stage. In midsummer the pupal stage, as observed in the laboratory, showed an average of 6.5 days. The shortest pupal period observed was 6 days, and the longest pupal period, not considering hibernation in that stage, was 35 days.

ADULT

EMERGENCE.—The adult (Pl. 78, *C*) upon emerging from the pupal skin finds itself completely inclosed within the walls of the alfalfa seed, which has been hollowed out by its host, *B. funebris*. It gnaws a small hole through the seed wall and through the seed pod, making its escape.

RELATIVE PROPORTION OF SEXES.—In connection with the rearing of adults of this species from different localities throughout California and western Arizona, it was found that both sexes were well represented, but that the females were considerably more abundant than the males. A total of 1,249 adults examined showed 210 to be males and 1,039 to be females, or a ratio of 1 to 4.94.

SEASONAL HISTORY

There are from two to four generations of this species in a single season, where conditions are favorable, as is the case in the alfalfa seed fields of central California. Under the less favorable, hot and dry desert conditions the species frequently has only a single generation in a season. The development from egg to adult of each generation is completed within the unbroken alfalfa seed infested by its host.

IMPORTANCE AS A PARASITE

Tetrastichus bruchophagi is a parasite of considerable importance in controlling *Bruchophagus funebris* in the alfalfa seed-growing districts of central California, where it apparently destroyed about 52 per cent of the chalcis-fly larvæ infesting alfalfa seeds in 1913.

In the alfalfa seed fields of the Yuma, Ariz., district this parasite is apparently of little importance at the present time. Observations show that it destroys only about 1.5 per cent of the chalcis-fly larvæ in that seed district.

PLATE 78

Tetrastichus bruchophagi

A.—Larva.
B.—Pupa.
C.—Adult.

Tetrastichus bruchophagi
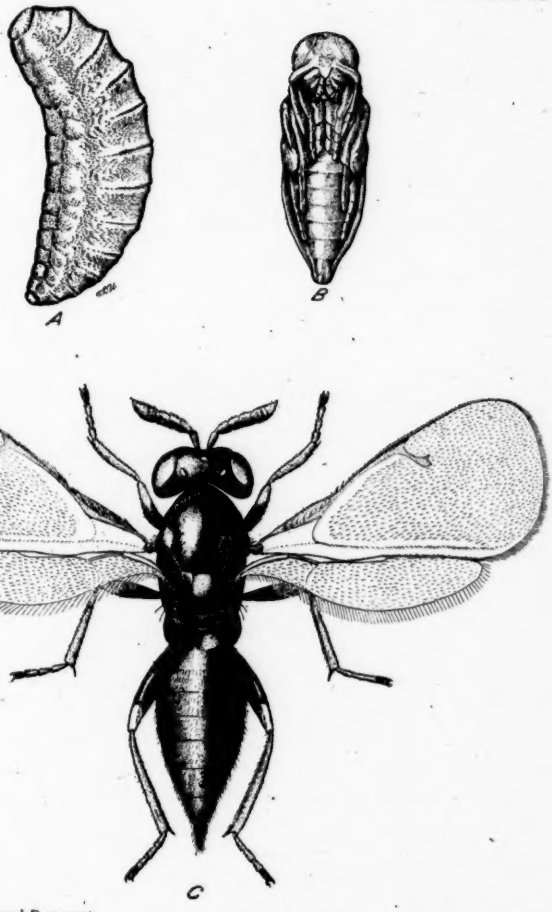
PLATE 78